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OF
LOW DOSES OF HYDROGEN SULFIDE

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Hydrogen sulfide exposure alters the amino acid content in developing rat CNS

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Hydrogen sulfide is a widespread environmental pollutant that can have severe effects on the nervous system. Neonatal rats were exposed continuously to hydrogen sulfide and amino acid concentrations were determined to establish the effects of exposure on the developing central nervous system. Results presented here demonstrate that amino acid concentrations are altered in the developing rat CNS. With an exposure regimen that mimics aspects of occupational exposure, the amino acid content was altered by hydrogen sulfide. The purpose of this study was to examine the effects of prolonged exposure to low amounts of hydrogen sulfide on amino acid concentrations in the developing mammalian brain.

Hydrogen sulfide (H_2S) is a colourless gas, well known and identified. As a serious environmental pollutant, it is a by-product of many industrial processes.

The available literature on H_2S points to the central nervous system as the major target for toxic effects. Little information exists on the effects of sublethal or passive effect on the developing organism.

The purpose of this study was to examine the effects of prolonged exposure to low amounts of hydrogen sulfide on amino acid concentrations in the developing mammalian brain.

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Hydrogen sulfide exposure alters the amino acid content in developing rat CNS

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Hydrogen sulfide is a widespread environmental pollutant that may produce severe effects on the developing nervous system. Putative amino acid neurotransmitter levels in the rat cerebrum and cerebellum were determined to evaluate the effects of exposure to hydrogen sulfide during perinatal development. The levels of aspartate, GABA, glutamate, glycine and taurine were quantitated using high-performance liquid chromatography. With the exception of glycine, all of the amino acids examined were affected by the treatment. On day 21 postnatal, which was the last day of the exposure, aspartate, glutamate and GABA in the cerebrum and aspartate and GABA in the cerebellum were significantly depressed. The observed alterations in the amino acid levels during this critical phase of development may have chronically affected the activity of the neurotransmitters, their receptor sensitivity or their individual target areas. The consequence of one or a combination of such alterations may lead to behavioral and structural abnormalities.

Hydrogen sulfide (H_2S) is an environmental and industrial pollutant. At high concentrations it is highly toxic and often fatal. H_2S gas is released into the atmosphere as a by-product of many industries, such as petrochemical refineries and plants, in addition to the numerous natural sources such as petroleum and natural gas wells [1].

The available literature on H_2S toxicity in the central nervous system has emphasized the high dose acute effects in the adult. As an example, Lund and Wieland [9] exposed rhesus monkeys to H_2S (500 ppm) for 20 min and reported necrosis in the cerebral cortex and a reduction in cerebellar Purkinje cells. Little information exists on the effects of subchronic exposures either on the adult or on the developing organism.

The purpose of this study was to examine the effects of prolonged exposure to low concentrations of H_2S on several putative amino acid neurotransmitters in the developing mammalian brain using the cerebrum and cerebellum as representative models.

Various amino acids have been identified as putative neurotransmitters in the rat cerebrum and cerebellum [11, 13]. Therefore, acute or chronic alterations in the levels of these amino acids during the development of the CNS may have severe ramifications on the growth process.

Timed-pregnant Sprague–Dawley rats were utilized in this study. The pregnant dams were exposed to H_2S (75 ppm) for 7 h per day from Day 5 postcoitus until Day 21 postnatal (P) in a custom designed plexiglass environmental chamber. Control animals were maintained in the same conditions without the H_2S exposure. Dams were allowed to eat and drink ad libitum except during the 7 h per day exposure.

The environmental chamber with a 90 liter volume capacity, contained a 12 compartment circular stainless-steel cage. The total animal displacement did not exceed 5% of the total volume of the chamber. The chamber was designed to permit continuous observation of the animals and the recording of temperature, pressure, humidity and H_2S concentration. H_2S concentration was measured at the animal level with a digital GFG (model GMA-100) monitoring system capable of detecting a maximum concentration of 100 ppm. Room air was drawn into the chamber with a vacuum blower and mixed with certified H_2S (900 ppm in nitrogen). The mixture was then passed through an orifice plate to measure flow rate, then through a diffuser in the top of the chamber. The diffused gas was vented out of a double port at the bottom of the chamber. The oxygen concentration was maintained at 20–21% with an air flow sufficient to produce a complete chamber replacement every 3 min.

All pups were weighed and there was no significant difference in either whole body or brain weight. Randomly selected animals (8 treated and 8 control) were euthanized at each sample time (P 7, 14 and 21). The rats were decapitated and the cerebrum (anterior to the optic chiasm) and cerebellum were rapidly removed. The tissue was then immediately weighed and placed in pre-cooled methyl butane and stored at $-70°C$. The control and experimental rats were manipulated in parallel. In addition, the period between brain removal and freezing was identical for all animals. Selected amino acids were quantitated using precolumn fluorescence derivatization with o-phthaldialdehyde/2-mercaptoethanol reagent and reverse-phase high-performance liquid chromatography (HPLC) [8]. The liquid chromatograph consisted of two Waters 510 pumps, WISP 710B automatic injector, 740 integrator and a 721 programmable system controller. The mobile phases (10 mM phosphate, pH 4.5 and a 65%/35% mix of HPLC grade methanol and 10 mM phosphate) were pumped on a gradient through a mixing column (glass beads, 600 μm), guard column (C18 Corasil) and a Waters C18 RESOLVE column maintained at 28°C. The OPT-amino acid derivatives were detected fluoroscopically with a FS970 L.C. Fluorimeter (Kratos) equipped with a Corning 7-54 primary filter and a 418 nm cut-off secondary filter. The excitation wavelength was set to 335 nm. The amino acids were calculated with reference to standards and the data were statistically analysed with Student's t-test.

The control levels of the amino acids (Table I) correlate with other published findings, demonstrating an increased concentration of all amino acids with age with the exception of taurine which declines with age [2].

TABLE I

CONTENTS OF AMINO ACIDS IN THE CEREBELLUM AND CEREBRAL CORTEX OF CONTROL RATS

Data represent the means $\pm$ S.E.M. of 8 animals.

Age	Mean wet weight (μmol/g)				
	Aspartate	GABA	Glutamate	Glycine	Taurine
1 week					
Cerebellum	3.3 ± 0.4	1.7 ± 0.4	8.5 ± 0.4	3.5 ± 0.3	10.7 ± 0.5
Cerebrum	3.1 ± 0.1	1.9 ± 0.2	8.1 ± 0.3	4.3 ± 0.2	10.2 ± 0.8
2 weeks					
Cerebellum	3.3 ± 0.3	2.5 ± 0.4	8.7 ± 0.5	3.6 ± 0.2	8.8 ± 0.4
Cerebrum	3.5 ± 0.4	2.7 ± 0.2	8.4 ± 0.4	4.9 ± 0.5	7.8 ± 0.3
3 weeks					
Cerebellum	3.7 ± 0.4	3.8 ± 0.1	8.9 ± 0.9	3.9 ± 0.4	6.2 ± 0.7
Cerebrum	4.3 ± 0.2	3.3 ± 0.2	8.7 ± 0.6	5.7 ± 0.2	6.9 ± 0.4

The effect of H_2S exposure on selected amino acid levels in the cerebellum is demonstrated in Fig. 1. The levels of aspartate, glutamate and GABA were significantly reduced below control levels by P21. Glutamate levels were initially unchanged but were reduced by approximately 20% at P21. By P14, both aspartate and GABA levels were reduced by approximately 20% and remained at that level at P21. Taurine, which was initially elevated (25%), returned to control levels by P21. Glycine levels remained unaltered.
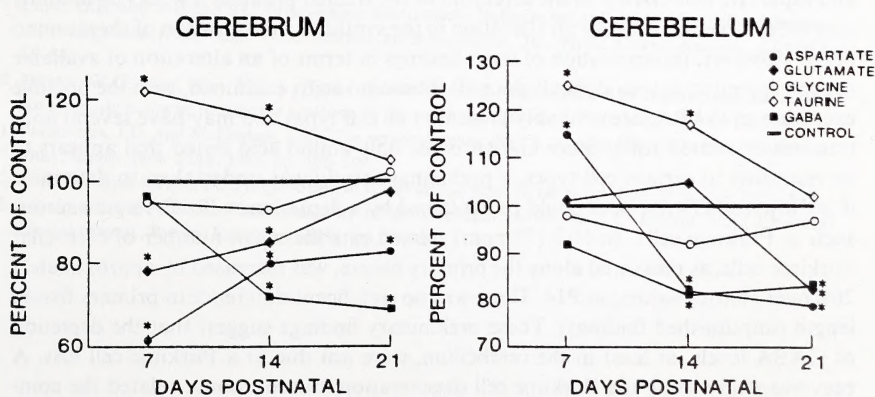

Fig. 1. Effects of hydrogen sulfide exposure on amino acid levels in the cerebrum and cerebellum. The relative amounts are presented in percent of the values in control animals of corresponding age. *$P < 0.05$

In the cerebral cortex (Fig. 1), levels of all of the amino acids tested with the exception of glycine were altered by the treatment on at least one sample time. Aspartate levels ranged from an approximate 40% reduction at P7 to a 20% reduction at P21. Glutamate was initially lower (20%) but recovered by P21. GABA levels were reduced by approximately 20% at both P14 and 21. As in the cerebellum, taurine levels were initially higher (20%) but fell to control levels by P21.

The high levels of the sulfur-containing amino acid taurine, observed up to and including P14 in both brain regions, suggest several possibilities. The neuromodulator taurine displays a characteristic decrease in concentration during the early stages of central nervous system development [7]. The decrease in the concentration of taurine occurs while the enzymatic activity of the taurine synthesizing enzymes and taurine transporting system increase [6]. During this same time the concentration of other neurotransmitters and neuromodulators increase. Alterations in taurine content have been observed with stress, mental retardation, muscle, liver and bone disease [5].

The elevated levels of taurine observed up to and including day 14 neonatally could be the result of enhanced synthesis from sulfate, through a pathway which has been demonstrated in rat liver [10]. Alternatively, the elevated taurine levels could be the result of increased metabolism of cysteine caused by a hydrogen sulfide-induced increase in protein metabolism. This increase in taurine would be similar to that observed with stress situations which cause an increased turnover of tissue protein and enhanced synthesis of taurine [5]. The return of brain taurine to normal levels on day 21 of development could be the result of the development of the blood brain barrier at P6–10 [7], or the development of the enzymatic capacity to metabolize taurine to isethionic acid [12] or the conjugation of taurine which is excreted in the bile [5].

The reduced levels of aspartate, glutamate and GABA observed in the cerebellum and aspartate and GABA in the cerebrum of the treated group at 3 weeks postnatally may reflect a cellular loss or an alteration in the synthesis or utilization of these amino acids. However, interpretation of these findings in terms of an alteration of available neurotransmitter is very difficult since all the amino acids examined, with the possible exception of GABA, are probably present in all cell types and may have several non-transmitter related roles. Since GABA is the only amino acid tested that appears to be restricted to certain cell types, a preliminary study was undertaken to determine if the lowered GABA pool could be explained by a depletion in GABAergic neurons such as Purkinje cells. In H_2S (75 ppm) treated rats the mean number of cerebellar Purkinje cells, as measured along the primary fissure, was increased by approximately 20% over control values, at P14. There was no significant difference in primary fissure length (unpublished findings). These preliminary findings suggest that the depletion of GABA levels, at least in the cerebellum, were not due to a Purkinje cell loss. A previous study using the Purkinje cell degeneration mutant mouse related the complete loss of Purkinje cells to a substantial reduction in the level of GABA. However, in a study using phenobarbital, perinatal treatment with phenobarbital produced a 20% reduction in Purkinje cell number [3] but the levels of GABA were increased by

40% [4]. It would therefore appear that other compensatory mechanisms may be involved and that care must be taken in interpreting fluctuations in amino acid levels in terms of cell numbers.

In summary, the prolonged exposure to a low concentration of H_2S, produced alterations in four of the five amino acids tested in both the cerebellum and cerebral cortex. The consequences of these shifts in the amino acid pool during this critical phase of brain development is as yet unknown but may result in long term behavioural problems.

1 Beauchamp, R.O., Bus, J.S., Popp, J.A., Boreiko, C.J. and Andjelkovich, D.A., A critical review of the literature on hydrogen sulfide toxicity, CRC Crit. Rev. Toxicol., 13 (1984) 25–97.
2 Cutler, R.W.P. and Dudzinski, D.S., Regional changes in amino acid content in developing rat brain, J. Neurochem., 23 (1974) 1005–1009.
3 Hannah, R.S., Roth, S.H. and Spira, A.W., The effects of chlorpromazine and phenobarbital on cerebellar Purkinje cells, Teratology, 26 (1982) 21–25.
4 Hannah, R.S., Roth, S.H. and Spira, A.W., The effects of two anticonvulsants on amino acid levels in the developing rat cerebellum, Neurochem. Res., in press.
5 Jacoben, J.G. and Smith, L.H., Biochemistry and physiology of taurine and taurine derivatives. In J.R. Brobeck (Ed.), Physiol. Rev. Am. Physiol. Soc., Bethesda, 1968.
6 Kishi, M., Ohkuma, S., Kimor, M. and Kuriyama, K., Characteristics of taurine transport system and its developmental pattern in mouse cerebral cortical neurons in primary culture, Biochim. Biophys. Acta, 939 (1988) 615–623.
7 Kuriyama, K., Ohkuma, S., Kishi, M. and Kimori, M., Development of biosynthesizing and uptake systems for taurine in cerebral cortical neurons in primary culture: Analysis of possible factors involved in perinatal decline of cerebral taurine, Adv. Exp. Med. Biol., 217 (1987) 69–77.
8 Lindroth, P. and Mopper, K., High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with o-phthaldialdehyde, Anal. Chem., 51 (1979) 1667–1674.
9 Lund, O.E. and Wieland, H., Pathologisch-anatomische Befunde bei experimenteller Schwefelwasserstoff-Vergiftung (H_2S). Eine Untersuchung an Rhesusaffen, Int. Arch. Gewerbepathol., 22 (1966) 46–54.
10 Martin, W.G., Sass, N.L., Hill, L., Tarka, S. and Truet, R., The synthesis of taurine from sulfate IV. An alternate pathway for taurine synthesis in the rat, Proc. Doc. Exp. Biol. Med., 141 (1972) 632–633.
11 Parnavelas, J.G. and McDonald, J.K., The cerebral cortex. In P.C. Emson (Ed.), Chemical Neuroanatomy, Raven, New York, 1983, pp. 505–549.
12 Peck, E.J. and Awapara, J., Metabolism of sulfur amino acids in rat brain, Fed. Proc., 25 (1966) 642.
13 Schulman, J.A., Chemical neuroanatomy of the cerebellar cortex. In P.C. Emson (Ed.), Chemical Neuroanatomy, Raven, New York, 1983, pp. 209–228.

INHIBITION OF OXYTOCIN-INDUCED BUT NOT ANGIOTENSIN-INDUCED RAT UTERINE CONTRACTIONS FOLLOWING EXPOSURE TO SODIUM SULFIDE

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Summary

Low concentrations (0.15-15 μM) of sodium sulfide reversibly attenuated the contractile response of the isolated rat uterus to oxytocin without affecting angiotensin II responsiveness. These findings suggest that functionally important disulfide bonds in the rat uterine oxytocin receptor, but not the angiotensin receptor, are sensitive to hydrosulfide ion. Reduction of oxytocin receptors by hydrosulfide ion may be a mechanism by which low levels of H_2S delay parturition in rats.

Sub-chronic exposure of gravid Sprague-Dawley rats to low concentrations of hydrogen sulfide (H_2S) produces a dose-dependent extension of litter delivery time at parturition (1). This dystocic effect following exposure of animals to 20, 50 and 75 ppm H_2S for 6 hours per day, every day from day 6 of gestation until term (21 days), is similar to the dystocia observed in rats administered acetylsalicylic acid (2). The delay in parturition time observed at these sub-chronic exposure levels of H_2S could cause complications at delivery in animals exposed to industrially or agriculturally generated H_2S. These complications could increase the number of asphyxiations in the birth canal and cause failure to deliver at term (1). Several mechanisms of action for H_2S-induced dystocia are possible. These include 1) disruption of uterine contraction by inhibition of energy production secondary to inactivation of cytochrome oxidase or other enzymes involved in energy production 2) inhibition of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) synthesis (as with salicylates) and 3) interference with the oxytocin induction of labor by direct action at disulfide bonds of either hormone or receptor complex, producing compromised ligand-receptor interaction. At lethal levels H_2S is believed to bind the ferric iron of the heme prosthetic group thereby inhibiting the final step in electron transport and energy production (4). It is also possible that low levels of H_2S inhibit this pathway by reducing the ATP levels required for optimum muscle contraction. Prostaglandin $F_{2\alpha}$ levels in uterine muscle have been shown to be elevated in term and preterm labor (3). This estrogen-driven elevation in $PGF_{2\alpha}$ is believed to prepare the uterine tissue for parturition by increasing the sensitivity of uterine muscle to oxytocin (5). Salicylate administration to rats at term suppresses the production of $PGF_{2\alpha}$ causing the observed dystocia (2), which was similar to that observed with low doses of H_2S (1). Sensitivity of uterine muscle to oxytocin could also be suppressed by chemical modification of either the disulfide containing hormone or its receptor. Previous studies have shown that oxytocin and oxytocin receptor are inactivated by mercapto-type reducing compounds (6).

The present study was designed to determine if H_2S could directly inhibit uterine muscle contraction in vitro, and to determine if H_2S disruption of oxytocin-induced contractions could be consistent with an action at the receptor level.

Methods

Rat uterine muscle was prepared as previously described (7). Oxytocin sensitivity was optimized by injecting virgin Sprague-Dawley rats (150-250 g) with 25 μg diethylstilbestrol in 0.05 ml of 20% polyvinyl pyrrolidone 24 hours before sacrifice and exsanguination. The uterine horns were dissected free of adherent fat, bisected, and each of the four tissues were suspended in a 3 ml tissue bath at 29°C in DeJalons solution which was continuously gassed with oxygen. Contractions were recorded by means of isotonic transducers against a load of 1 g. The preparation was allowed to stabilize for one hour before responses to oxytocin or angiotensin II were established. ED_{50} doses for oxytocin and angiotensin II were determined to be 0.2 nM and 1.5 nM respectively and these concentrations were employed throughout these studies as the reference concentration for each hormone (defined in Fig. 1 as 100% of the measured response).

Sodium sulfide was dissolved in oxygenated DeJalons solution. Na_2S concentration was established using an Orion SX-1 sulfide electrode and then diluted to the final concentration (0.15-15 μM) with DeJalons buffer just before tissue exposure began. Tissues were incubated with various concentrations of Na_2S in DeJalons solution for ten minutes without further oxygenation (in order to avoid purging of the volatile H_2S formed) and the Na_2S-containing buffer was washed from the chamber with 2 volumes of DeJalons buffer. Oxygenation was restarted and tissues were immediately challenged with oxytocin or angiotensin II. An angiotensin II control was included in these studies because the rat angiotensin II receptor appears to be resistant to the reducing power of mercaptides (8) and could provide evidence for the viability of the tissue. Control experiments were identical to the experimental conditions except for the exclusion of Na_2S; tissues were incubated for ten minutes without continuous oxygenation, followed by oxytocin or angiotensin II challenge with oxygenation. Tissues stimulated with oxytocin were exposed to 0.15, 1.5 and 15 μM Na_2S for ten minutes before stimulation, and tissues stimulated with angiotensin II were exposed to 0.15 μM Na_2S for ten minutes before stimulation. The reversibility of Na_2S inhibition of uterine contractions was determined at various times following the removal of the DeJalons buffer containing Na_2S. Exposed tissues were challenged with oxytocin or angiotensin II immediately after removal of Na_2S and at five and ten minutes after removal of Na_2S. The contractile response of each muscle after exposure to Na_2S was determined as a percent of the standardized ED_{50} response to each hormone. Values from various concentrations of Na_2S were compared by analysis of variance (ANOVA) and the Student-Newman-Keuls multiple comparison test; p values of less than 0.05 were considered significant.

Results

Muscle preparations from four different animals were employed for these studies. Incubation of the uterine muscle in DeJalon's buffer without continuous oxygenation for 10 min produced approximately a 10% reduction in contraction to either hormone (Fig. 1) and were included to demonstrate that the assay conditions did not significantly interfere with the response. As shown in figure 1, a similar response to angiotensin II was obtained in

unexposed tissues and in tissues exposed to 0.15 µM H$_2$S, demonstrating that
the muscle remained viable and continued to contract at an amplitude similar
to the control. Exposure to 0.15, 1.5 and 15 µM Na$_2$S for ten minutes caused a
marked reduction in the strength of oxytocin-induced muscle contractions (Fig.
1) at all concentrations of Na$_2$S. There was no significant difference between
the strength of contraction at the various doses used in this study,
suggesting that it may be necessary to expand the exposure concentration range
in order to establish the sensitivity of these muscle preparations to Na$_2$S.
Na$_2$S inhibition of oxytocin-induced contraction is rapidly reversible at all
concentrations analyzed. Immediate challenge of exposed uterine tissue to
oxytocin demonstrated that uterine contractions were attenuated by 40-60%.
However, upon repetition of the oxytocin challenge at 5 and 10 minutes post
washout of the Na$_2$S, a second and third oxytocin stimulation produced the
pretreatment muscle contraction.
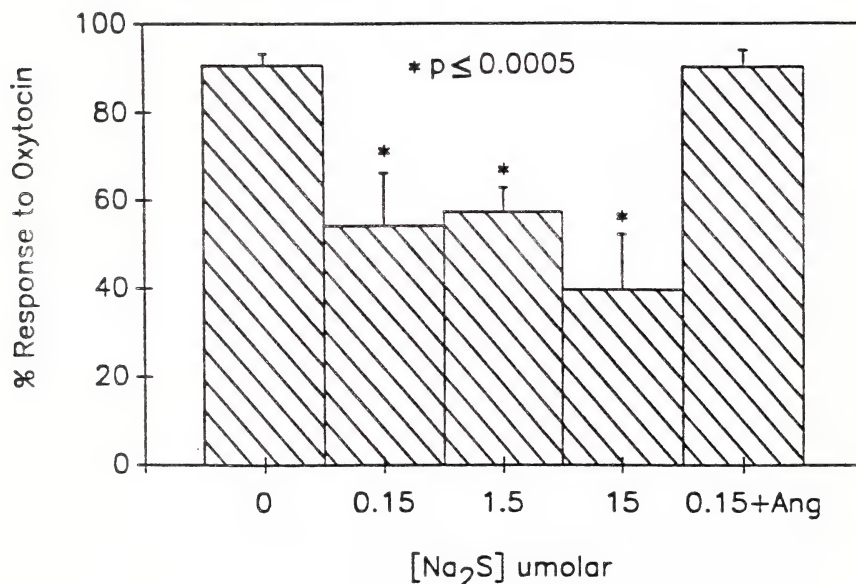
FIG. 1
Oxytocin and angiotensin II induced uterine contractions following
varying doses of Na$_2$S. Values are presented as the percent of the
ED$_{50}$ for each ligand.

Discussion

In aqueous solutions, Na$_2$S is rapidly converted to hydrogen sulfide and
thence hydrosulfide ion. The hydrosulfide ion product of H$_2$S and water is a
potent reducing agent which acts to break disulfide bonds in a reversible
manner. In this study, Na$_2$S was found to directly inhibit uterine muscle
contractions at a concentration range which produces levels of H$_2$S similar to
that anticipated to be present in animals exposed to low levels of H$_2$S (1).
Since Na$_2$S specifically inhibits oxytocin-induced contractions without

affecting angiotensin II-induced contractions, it would appear that
hydrosulfide ion or one of the oxidation products of H_2S may act directly on
the oxytocin receptor. If the site of inhibition was post-receptor, e.g.
inhibition of energy production or inactivation of common post receptor
messengers, then both angiotensin II and oxytocin responsiveness would be
simultaneously affected. Although oxytocin contains disulfide bonds and could
potentially be compromised by Na_2S, in this study oxytocin was not exposed to
Na_2S and therefore could not be the site of inhibition.

The oxytocin receptor contains both free thiol groups and disulfide bonds
which are involved in ligand binding (6). Thus reduction of the oxytocin
receptor with dithiothreitol inactivates high affinity binding sites for
oxytocin, while blockade of the free thiol groups with N-ethylmaleimide causes
even greater inhibition of oxytocin binding (6). In similar studies it has
been shown that the rat uterine angiotensin II receptor is not sensitive to
thiol reducing agents and retains its binding characteristics in the presence
of dithiothreitol. The similarity of response of rat tissue to Na_2S and
dithiothreitol suggests that a similar mechanism of action is involved.
Alternatively, hydrosulfide ion may disrupt disulfide bonds in non-receptor
membrane proteins causing a reduction in oxytocin-induced contractions.
Direct characterization of receptor binding in the presence of Na_2S will be
required to document the interaction between receptor and hydrosulfide ion.
The present findings suggest hydrosulfide causes loss of oxytocin
responsiveness by reducing susceptible receptor-based disulfide bonds in a
reversible manner. The reversibility of the effects of Na_2S on oxytocin
receptors supports the involvement of hydrosulfide ion, rather than higher
oxidation products such as sulfite which react irreversibly. The present
findings, may, at least in part, explain the dystocic effect of H_2S observed
in vivo.

Acknowledgements

References

1.   K.J. FRANKLIN, L.J. HAYDEN, S.H. ROTH and G.J. MOORE, Proc. Can. Fed.
      Biol. Soc. 32 11 (1989); manuscript submitted for publication.
2.   J.W. AIKEN, Nature 240 21-25 (1972).
3.   J.B. LEE, Text Book of Endocrinology, Robert H. William (ed), 1047-1063
      (1981).
4.   R.O. BEAUCHAMP, J.S. BUS, J.A. POPP, C.J. BOREIKO and D.A. ANDJELKOVICH,
      CRC Rev. of Toxicology 13(1) 25-67 (1984).
5.   R.J. FLOWER, A Ciba Foundation Symposium: The Fetus and Birth, 47,
      297-318, 1977.
6.   D. BOLAND and H.J. GOREN, Reg. Pept. 18 7-18 (1987).
7.   M.N. SCANLON and G.J. MOORE, J. Pharmacol. Methods 20 207-224 (1988).
8.   M.N. SCANLON, P. KOZIARZ and G.J. MOORE, Gen. Pharmacol. in press (1989).

A RELATIONSHIP BETWEEN HYDROGEN SULFIDE EXPOSURE AND TAURINE LEVELS IN MATERNAL RATS

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Hydrogen sulfide is an environmental and industrial pollutant. The effects of prolonged exposure to low concentrations is not well established, however, it has been described as a form of chronic poisoning (1).

We have previously demonstrated that exposure to low concentrations of hydrogen sulfide (H_2S) produces a significant increase in the level of taurine in the developing rat CNS which return to control values at the same approximate time as the establishment of the blood-brain barrier (1). The purpose of this study was to establish if this observed increase was the result of increased maternal plasma levels of taurine.

METHOD: Timed-pregnant Sprague-Dawley rats were utilized in this study. The pregnant dams were exposed to hydrogen sulfide (50 ppm) for seven hours per day from Day 6 postcoitus until Day 21 postnatal (pn) in a custom designed plexiglass environmental chamber. Control animals were maintained in the same conditions without the H_2S exposure. Dams were allowed to eat and drink ad libitum except during the seven hour per day exposure. The environmental chamber with a 90 liter volume capacity, contained a 12 compartment circular stainless steel cage. The total animal displacement did not exceed five percent of the total volume of the chamber. The chamber was designed to permit continuous observation of the animals and the recording of temperature, pressure, humidity, and hydrogen sulfide concentration. Hydrogen sulfide concentration was measured at the animal level with a digital GFG (model GMA-100) monitoring system capable of detecting a maximum concentration of 100 ppm. Room air was drawn into the chamber with a vacuum blower and mixed with certified hydrogen sulfide (900 ppm in nitrogen). The mixture was then passed through an orifice plate to measure flow rate, then through a diffuser in the top of the chamber. The oxygen concentration was maintained at 20-21% with an air flow sufficient to produce a complete chamber replacement every three minutes. Maternal blood plasma was sampled on the day of partuition and Day 21 pn. Taurine levels were quantitated using precolumn fluorescence derivitization with OPT and reversed-phase high performance liquid chromatography.

RESULTS-DISCUSSION: Taurine levels in maternal plasma were found to be increased at both sample times (Fig 1). In contrast, the brain levels of taurine in the pups had returned to control levels by day 21 pn. The observed reduction in CNS taurine levels in the pups coincides with the establishment of the blood-brain barrier to taurine. Since taurine is rapidly transferred from the dam to the pups both transplacentely and via the milk, (3) it is highly probable that the pup blood plasma levels of taurine were still elevated on day 21 pn after CNS levels had declined to normal values. Therefore, the high initial levels of taurine observed in the pup CNS may have been maternal in origin and not endogenously produced. The

abnormally high taurine levels initially present in the treated pup brains occurred at a time of maximum sensitivity of neuronal growth to both exogenous and endogenous factors. The excess taurine present during this phase of growth would have the potential to produce neuronal abnormalities.

Since taurine is also known to have an attenuating action on toxic compounds (4), the rise in maternal taurine levels may represent a protective mechanism driven by the H_2S exposure.

REFERENCES:

1.   Beauchamp, R.O., Bus, J.S., Popp, J.A., Boreiko, C.J. Andjelkovich, D.A.: CRC Crit. Rev. Toxicol. 13:25 (1984).
2.   Hannah, R.S., Hayden, L.J. & Roth, S.H.: Neurosci. Lett. 99:323 (1989).
3.   Sturman, J.A., Rassin, D.K. & Gaull, G.E.: Pediatr. Res. 11:29 (1977).
4.   Gaull, G.E., Pasantes-Morales, H. & Wright, C.E.: In Taurine: Biological Actions and Clinical Perspectives. (ed) S. Oja, Allan R. Liss, (1985), p. 3.
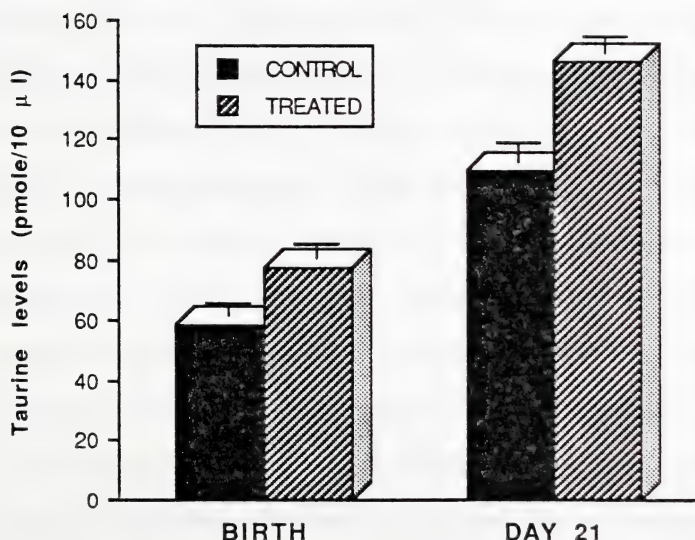
FIGURE 1:   Effects of low dose hydrogen sulfide exposure on maternal blood plasma levels of taurine. Data represents mean ±SEM

NORMAL GROWTH AND DEVELOPMENT IN THE RAT DURING SUB-CHRONIC EXPOSURE TO LOW LEVELS OF HYDROGEN SULFIDE

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The effects of low levels of hydrogen sulfide (H_2S) on mammalian growth and development are unknown although it has long been postulated that H_2S can inhibit critical developmental functions through the breakage of disulfide bonds and chealation of essential metal ions. Gravid rat dams exposed to H_2S ($\leq$ 75 PPM) from day 6 of gestation until day 21 postpartum (PP) demonstrated normal development of gestation until parturition. At parturition, however, the delivery time was extended in a dose dependent manner with a increase of 42% at 75 PPM. Maternal liver cholesterol content was elevated significantly on day 21 postpartum when the dam was exposed to 75 PPM H_2S each day for 6 weeks. Pups which were exposed in uteri and neonatally to day 21 postpartum developed normally with no significant change in growth and development through day 21 postpartum.

INTRODUCTION

The lethal consequences of high dose hydrogen sulfide (H_2S) have been known for some time. Hydrogen sulfide is believed to bind to metal-containing proteins causing cellular death by disruption of energy producing metabolism (Beauchamp et al., 1984). However, recent studies (Bariliak et al., 1975; Savolainen et al., 1980) suggest that acute poisoning could be the result of interactions at the cellular plasma membrane level which produce rapid neurological dysfunction and respiratory paralysis, possibly as a result of disruption of critical disulfide bonds or disruption of membrane lipids.

A limited number of animal studies suggest that acute and chronic exposure to low levels of H_2S caused eye damage (Nordstrum and McQuitty, 1975), lung pathology (Sandage, 1961), trachea and bronchi irritation (Duan, 1959), cardiac irregularities and depression of cardiac enzyme activity (Kosmider et al., 1967), changes in the hematopoietic system (Duan, 1959) and permanent alterations in neurological pathology and biochemistry (Sandage, 1961). Many of these studies have been carried out in combination with other environmental compounds which complicate the assignment of a toxic effect exclusively to H_2S. In a well controlled 90 day exposure of adult B_6C_3F mice and Fisher 344 and Sprague-Dawley adult rats, an extensive investigation was unable to demonstrate any changes in pathology, biochemistry or behavior except at 80 PPM H_2S (Chemical Industry Institute of Toxicology, 1983). At this higher concentration, exposed mice and rats exhibited a decrease in body weight, an increase in eye and skin

irritation, inflammation of nasal mucosa and a depression of brain weight in the Fisher 344 rats. Occupational or low levels of H_2S are reported to be associated with many complaints of fatigue, somnolence, lack of initiative, decreased libido, loss of appetite, headache, irritability, poor memory, anxiety, dizziness, itching, eye irritation, respiratory tract irritation, gastrointestinal disorders, insomnia and backache (Beauchamp et al., 1984). The effects of chronic H_2S exposure on growth and development of fetal animals or on maternal development during gestation have not been previously documented. Embryotoxicity and teratogenicity have been reported in rats exposed concurrently to 70 PPM H_2S and carbon disulfide (Bariliak et al., 1975) and when rats were treated with "thermal" mineral water (Beauchamp et al., 1984). The combination of H_2S with carbon disulfide, a known teratogen, complicates interpretation of the results. Limited evidence demonstrates that adult rats are more susceptible to growth inhibition by H_2S than 8 week old animals (Sinitsain, 1962).

This laboratory has been conducting a series of studies using the maternal and developing rat model to determine if low levels of H_2S ($\leq$ 75 PPM) cause an alteration in maternal health and fetal or neonatal growth and development. The period of development of the central nervous system and metabolic function continues into the neonatal period and has been well characterized in the developing rat. The accumulation of protein, DNA, RNA and cholesterol have been correlated with various stages of development and can be employed as indicators of normal growth and development. This period appears to be especially susceptible to toxic insult because of a lack of detoxification

mechanisms (Buelke-Sam and Kimmel, 1979) and because during this period many hormones and growth factors such as insulin and epidermal growth factor play critical roles in development and maintenance of normal physiology (Kaplan, 1983). The function of either of these disulfide-containing peptides or their receptors could be easily disrupted by H_2S, causing changes in growth or development. This report documents the maternal health and fetal and neonatal physical development following exposure from day 6 of gestation through day 21 post partum (PP).

METHODS

Male and female Sprague-Dawley rats 80-90 days of age were allowed to acclimatize for one week before pairs were cohabitated for a maximum of five days or until positive evidence of mating. Positive evidence of mating was the detection of sperm in the vaginal lavage and this occasion was designated as day 1 (G1) of gestation. All animals were housed in plastic cages and maintained on a 12 hour light-dark cycle with ad libitum food and water supplies except during the daily seven hour period of exposure to H_2S. Exposure concentrations were 20 PPM, 50 PPM and 75 PPM, a concentration range which is below the lethal levels and near possible exposure levels.

Gestating animals were exposed in an acrylic dome-shaped inhalation chamber, which was a modification of the design described by Laskin and Drew (1970). The chamber was approximately 90 litres in volume and contained a 12-compartment circular galvanized cage. The total animal volume did not exceed five percent of the total volume of

the chamber. The chamber allowed continuous observation of the animals, monitoring and recording of temperature, pressure, humidity, and H_2S concentration. Hydrogen sulfide concentration was measured at the animal's breathing level with a pre-calibrated digital GFG model GMA-100 monitoring system (Besellschaft für Gierätebau, Dortmund, West Germany) modified to measure a maximum concentration of 200 PPM.

Air supply to the chamber was room air drawn through a HEPA filter with a vacuum blower, mixed with certified H_2S (2000 PPM in nitrogen from Matheson Gas, Edmonton, Alberta, Canada) with the maintenance of an oxygen concentration of 20-21% and an airflow of 20 chamber air changes per hour. The exposure mixture was passed through an orifice plate to measure flow rates, then through a diffuser in the top of the chamber. Previous testing verified a homogeneous distribution of H_2S throughout the chamber. The exposure concentration was (± 5%) attained within 10 minutes of introduction of the gas. The diffused gas passed through the chamber and out a double port at the bottom of the chamber. H_2S and H_2O were trapped using sodium hydroxide solution and dry ice traps, respectively before venting into an exhaust duct.

Twenty gravid females were placed in two matched groups, maintained under identical conditions except for exposure of the experimental group to H_2S at concentrations of 20, 50 or 75 PPM. At parturition (Day 1 PP) each group was culled to 6 litters with 12 pups in each litter. Food and water consumption were monitored throughout gestation and maternal weights were observed until the dam was sacrificed on Day 21 PP. Parturition time was defined as the elapsed

time from the first sign of labor until the final pup was delivered. The male to female ratio and viability of pups at birth and throughout the neonatal exposure period were monitored.

Physical development of exposed and matched control pups was compared following the determination of the mean litter age for pinna detachment, incisor eruption, eyelid opening and growth. Pinna detachment was observed daily from Day 1 postpartum until both pinnae on all pups was detached and in the erect position. Incisor eruption was observed daily from Day 1 until both upper and lower incisors erupted in all litter pups. Eye opening was observed daily from Day 10 until both eyes of all pups were open (Savoleinen et al., 1980; Nordstrum and McQuitty, 1975). Pups were weighed on Days 1, 7, 14 and 21 PP.

Assessment of motor development was carried out using surface righting analysis (Vorhees, 1979). Surface righting was observed daily from Day 3 postpartum. Pups were tested until all animals in a litter could right themselves and plant their feet in less than two seconds in two trials on a given day.

Serum, liver and brain tissue samples were collected on Days 1, 7, 14 and 21 after delivery. Tissue samples were quick frozen on dry ice and stored at -70°C until analyzed. Serum samples were stored at 4°C and analyzed as soon as possible. Brain and liver samples were homogenized in 0.05 M Tris buffer, pH 7.0, containing 0.25 M sucrose. Aliquots were removed for protein analysis (Lowry et al., 1951),

perchloric acid extraction and analysis of DNA (Vytasek, 1982) and extraction and analysis of tissue cholesterol (Abell et al., 1952). Values were normalized to compare the content per gram of tissue.

Nonparametric data were analyzed using the Mann-Whitney U-test for significance and parametric data were compared using analysis of variance and Duncan's Multiple Range Test or Student-Newman-Keuls procedure. A P value of ≤ 0.05 was considered significant.

RESULTS

Maternal body weight gain was similar for both the control dams and the exposed dams (Table 1) with a weight gain for each of approximately 58% of the initial weight on the first day of gestation (G1). Food intake (Table 2) was suppressed during the first 4 days of exposure to 50 PPM H_2S and for the first 8 days of exposure to 75 PPM H_2S. This suppressed appetite then returned to normal or to slightly elevated values. Water intake (Table 2) was not significantly altered. Analysis of maternal organ and body weight demonstrated that there were no significant changes in either brain or liver weight due to the decrease in food intake (Table 3). At the exposure levels used the length of gestation, viability, litter size or male to female ratio of newborn pups were not altered.

(Tables 1,2,3)

The most significant departure from normal gestational events or behavior displayed by the dams in this study was the increase in parturition time. As shown in Figure 1 there is a dose dependent

increase in parturition time with an increase of 10,20 and 42 percent at 20,50 and 75 PPM respectively. The mean delivery time for the control animal was 96.8 minutes.

(Figure 1)

Neonatal pup body weights were not significantly different from matched control animals through day 21 of development (Table 4). Brain weight and liver weights of exposed pups were not significantly different from the controls, with no difference in organ to body weight ratios (Table 4). When normalized by organ weight, protein, DNA and cholesterol accumulation in pup brain (Table 6) or liver (Table 5) through day 21 PP were not significantly changed from the controls. However, it should be noted, the cholesterol content of maternal liver and brain were elevated at 75 PPM. The liver elevation of cholesterol was found to be statistically different. In these studies neither pup cholesterol nor DNA content had attained the mature adult levels by day 21 PP of development in either tissue, and the pup liver protein content on day 21 PP was lower than that of the dam. Total liver and brain DNA continued to accumulate through day 21 PP indicating that cellular replication was continuing to develop normally.

(Tables 4,5,6)

Pinna detachment, hair development, incisor eruption, eyelid opening and surface righting are sensitive parameters of development (Aldur, 1983). Each of these parameters was monitored and as shown (Table 3) there were no significant differences between the control and exposed pups.

DISCUSSION

In this study the animals were exposed to low levels of H_2S from day 6 of gestation until day 21 PP, a time at which the pups are usually weaned from the dam and the point in development when cell replication is complete (Fish and Winick, 1969). A decrease in maternal food intake was noted at the time of initial exposure of the dam to 50 and 75 PPM H_2S, followed by recovery of appetite after several days (Table 2). This reduction of food intake did not appear to cause a change in weight gain in the dams during gestation (Table 1). The data were in agreement with similar studies using calves exposed to 20 PPM H_2S, in which the animals showed signs of lethargic activity and general discomfort, but recovered after 7 days (Nordstrum and McQuitty, 1975). This response may be similar to the response in humans after subacute toxic exposure and indicates that the animals have adjusted to the exposure with possible induction of metabolic enzymes necessary to detoxify H_2S.

Exposure of the dams to low levels of H_2S after fertilization and implantation did not cause a significant change from matched controls in pup or maternal brain or liver weight, length of gestation, litter size, pup birth weight, male to female pup ratio or viability (Table 3). These values for gestational and fetal development are similar to the values established over many generations (Lang, 1988) except for control and exposed pup birth weights which were almost double that expected. Maternal levels of cholesterol were elevated in liver and possibly in brain samples after the dam had been exposed to 75 PPM H_2S

for approximately 6 weeks although there was no change in pup cholesterol levels. As suggested by earlier studies (Sinitsain, 1962), adult rats appear to be more susceptible to H_2S insult possibly because of slowed metabolism or protein synthesis. Our observation that dams exposed to 75 PPM H_2S continued to gain weight normally is in marked contrast to similar studies in both male and female Sprague-Dawley rats carried out over 90 days (Chemical Industry Institute of Toxicology, 1983).

The mean parturition time was extended at all levels of exposure and appeared to be dose dependent. However, not all dams demonstrated an extension of the labor time. This response was animal dependent with many of the animals having a normal length of labor, but the remaining animals having extended and difficult deliveries. In a few instances this extended labor time resulted in fetal death due to asphyxiation. Similar prolongation of labor has been observed in rats administered acetylsalicylic acid (Tuchmann-Duplessis et al., 1975). The salicylic-induced dystocia response was the result of a suppression of prostaglandin $F_{2\alpha}$ production. Although it is possible that prostaglandin production was directly modulated by H_2S, preliminary evidence from this laboratory suggests that the oxytocin receptor may be compromised (Hayden et al., 1989). The mechanism for the prolongation of labor could be the result of one or a combination of the following factors: disruption of disulfide bonds in oxytocin or the oxytocin receptor molecule, disruption of prostaglandin synthesis or inhibition of energy production at the electron transport level. Studies of the reproductive response to H_2S exposure alone do not

exist, although a single study reported embryotoxicity when H_2S and CS_2 were administered simultaneously to rats (Nordstrum and McQuitty, 1975). No post implantation toxicity was observed in the present study although a decrease in viable births is possible because of the dystocia. Multigeneration studies will be necessary to determine the effect of H_2S on pre and post-implantation toxicity as well as fertility.

Analysis of organ weight gain, protein, DNA and cholesterol accumulation as an index of growth and development indicated that the rat pups exhibited a normal growth pattern similar to that previously documented. None of the values were significantly different from the matched controls. Pup brain and liver continued to accumulate DNA throughout the observation period without any deviation from normal control values. This period of DNA synthesis and cellular replication is a sensitive period in which cells are subject to damage by mutation or alteration in control of growth. Neither DNA content nor cholesterol content in the pups at day 21 PP had matured to the adult level, therefore, it is possible that a major alteration in growth could occur as the animals mature beyond day 21 PP.

In summary, low levels of H_2S during gestation and neonatal development to day 21 PP does not alter observed fetal development. However, H_2S at all levels monitored extended parturition time and possibly elevated cholesterol content of the maternal liver and brain.

ACKNOWLEDGEMENTS

REFERENCES

ABELL, L.L., LEVY, B.B., BRODIE, B.B. and KENDELL, F.E. (1952). A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J. Biol. Chem. 195:357-366.

ALDUR, S. (1983). In: Application of Behavioral Pharmacology in Toxicology (G. Zbinden, et al. eds.), Raven Press, New York.

BARILIAK, I.R., VASILEVA, I. and KALINOVSHEJA, L. (1975). Effect of small concentrations of carbon disulfide and hydrogen sulfide on the intrauterine development of rats. Arkh. Anato. Gistol. Embriol. 68(5):77-81.

BEAUCHAMP. R.O., BUS, J.S., POPP, J.A., COREIKO, C.J. and ANDJELKOVICH, D.A. (1984). A critical review of the literature on hydrogen sulfide toxicity. CRC Review of Toxicology 13(1):25-67.

BUELKE-SAM J. and KIMMEL, A. (1979). Development and standardization of screening methods for behavioral teratology. Toxicology 20:17-30.

CHEMICAL INDUSTRY INSTITUTE OF TOXICOLOGY (CIIT) (1983). 90 day vapor inhalation toxicity study of hydrogen sulfide in B_6C_3F mice, Fisher 344 rats and Sprague-Dawley rats.

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DUAN, F.Z. (1959). Maximum permissible concentration of hydrogen
    sulfide in the atmosphere. Gig. Sanit. 24(10):12-17.

HAYDEN, L.J., FRANKLIN, K.J., ROTH, S.H. and MOORE, G.J. (submitted to
    Life Science). Inhibition of oxytocin-induced but not angiotensin-
    induced rat uterine contractions following exposure to sodium
    sulfide.

FISH, I. and WINICK, M. (1969). Cellular growth in various regions of
    the developing rat brain. Pediat. Res. 3:407-412.

KAPLAN, S.H. (1983). Ontogenesis of hormone action: Insulin, adrenal
    corticoids, thyroid hormones, and growth hormone. In: The
    Biological Basis of Reproductive and Developmental Medicine (J.B.
    Warshaw, ed.), pp. 289-300, Elsevier, New York.

KOSMIDER, S., ROGALA, E. and PACHOLEK, A. (1967). Electrocardiographic
    and histochemical studies of the heart muscle in acute
    experimental hydrogen sulfide poisoning. Immun. Ther. Exp.
    15:731-740.

LANG, P.L. (1988). Embryo and fetal developmental toxicity (teratology)
    control data in the Charles River Crl:CD BR rat. Charles River
    Publication 1-23.

LASKIN, S. and DREW, R.T. (1970). An inexpensive portable inhalation
    chamber. Ann. of Industrial Hyg. 31:645-646.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. and RANDALL, R.J. (1951).
    Protein measurement with the folin phenol reagent. J. Biol. Chem.
    193:265-275.

NORDSTRUM, G.A. and McQUITTY, J.B. (1975). Response of calves to
    atmospheric hydrogen sulfide and ammonia. Proc. Can. Soc. Agric.
    Eng. 75-212.

SANDAGE, C. (1961). Tolerance criteria for continuous inhalation exposure to toxic material. II. Effects on animals of 90 day exposure to H_2S methyl mercaptan, indole and skatole. Armed. Ser. Tech. Info. Agency (ASTIA AD 287797).

SAVOLAINEN, H., TENHUNEN, R., ELOVAARA, E. and TOFFAVAINEN, A. (1980). Accumulative biochemical effects of repeated subclinical hydrogen sulfide intoxication in mouse brain. Int. Arch. Occup. Environ. Health 47:87-92.

SINITSAIN, S.N. (1962). Age of rats and sensitivity to hydrogen sulfide. Farmakol. Toksikol. 25:232-240.

SMART, J.L. and DOBBING, J. (1971). Vulnerability of developing brain. II. Effects of early nutritional deprivation on reflex ontogeny and development of behaviour in rat. Brain Res. 28:85-95.

TUCHMANN-DUPLESSIS, H., HISS, D., MOTTOT, G. and ROSNER, I. (1975). Effects of prenatal administration of acetylsalicylic acid in rats. Toxicology 3:207-211.

VORHEES, C.V., BUTCHER, R.E., BRUNNER, R.L. and SOBOTKA, T.J. (1979). A developmental test battery for neurobehavioral toxicity in rats: A preliminary analysis using monosodium glutamate, calcium, carrageenan and hydroxyurea. Tox. Appl. Pharm. 50:267-282.

VYTASEK, R. (1982). A sensitive fluorometric assay for the determination of DNA. Anal. Biochem. 120:243-248.

WINICK, M. and NOBLE, A. (1965). Quantitative changes in DNA, RNA and protein during prenatal and postnatal growth in the rat. Developmental Biology 12:451-466.

FIGURE LEGEND

Figure 1: Parturition time presented as the percent of matched
controls. Gravid dams were exposed to H_2S for seven
hours per day from day 6 of gestation until term.
Values are the mean ± SE for all dams observed at each
H_2S concentration.

Table 1

Maternal Body and Tissue Weight (gm) During Exposure to Hydrogen Sulfide

Day	Control	Dose 20 PPM	50 PPM	75 PPM
G1[a]	231.0 ± 7.2(24)	229.5 ± 7.4(12)	226.9 ± 8.8(6)	220.0 ± 11.6(6)
G5	252.0 ± 7.3	249.5 ± 7.0	244.8 ± 8.9	237.9 ± 11.6
G9	262.5 ± 8.4	261.1 ± 8.3	251.5 ± 8.6	244.8 ± 12.4
G13	280.0 ± 8.7	262.0 ± 11.8	268.4 ± 8.5	283.2 ± 10.4
G17	305.7 ± 9.4	307.6 ± 9.2	295.7 ± 10.0	289.6 ± 12.4
G21	365.0 ± 13.4	367.4 ± 9.0	359.4 ± 9.0	348.2 ± 12.09
PP 1[b]	285.4 ± 12.5	283.2 ± 12.4	267.2 ± 12.4	267.2 ± 15.0
PP 7	301.3 ± 10.5	305.7 ± 12.4	317.8 ± 23.2	276.82 ± 9.94
PP 14	324.1 ± 8.0	321.5 ± 11.7	306.6 ± 10.1	300.4 ± 11.3
PP 21	296.4 ± 7.3	289.4 ± 9.6	283.0 ± 5.3	271.03 ± 9.9
Liver Weight	54.3 ± 2.0	51.3 ± 3.0	52.9 ± 1.7	54.0 ± 2.5
Brain Weight	5.8 ± .4	5.9 ± .3	6.2 ± .5	5.9 ± .6

Data was the mean ± SE

( ) = number of samples

[a] lM/gm body weight

[a]$_G$ = day of gestation

[b]$_{PP}$ = day postpartum

Table 2

Maternal Food and Water Intake During Gestation

Collection Interval (Days)

	5-9	9-13	13-17	17-21
Dose	Food consumption (gm/gm Body Weight)			
Control	.303 ± .005(24)	.281 ± .004	.269 ± .003	.262 ± .003
50 PPM	.270 ± .005*(6)	.296 ± .007	.287 ± .004	.272 ± .005
75 PPM	.262 ± .004*(6)	.259 ± .004*	.262 ± .008	.260 ± .006

Water Consumption (ml/gm Body Weight)

	5-9	9-13	13-17	17-21
Control	.61 ± .02	.62 ± .02	.65 ± .02	.66 ± .03
50 PPM	.56 ± .03	.61 ± .03	.67 ± .04	.65 ± .03
75 PPM	.59 ± .02	.66 ± .01	.63 ± .02	.64 ± .02

Values are the mean food or water consumed ± SE.

*Significantly different from control p ≤ 0.05.

( ) number of litters in each dose group

Table 3

Developmental and Reproductive Data

Parameter	Control	Dose 20 PPM	50 PPM	75 PPM
Gestation Length (Days)	22.4 ± .4(24)	22.6 ± .5(12)	22.3 ± .5(6)	22.3 ± .5(6)
Litter Size	13.5 ± .7	13.1 ± .8	14.1 ± .9	14.0 ± .4
Litter Viability[a]	94.6 ± 5.5	96.8 ± .2	99.0 ± .8	96.5 ± 1.2
Male/Female Ratio	1.4 ± .2	1.1 ± .2	1.2 ± .2	1.1 ± .1
Pinna Detachment[b]	4.9 ± .2	4.5 ± .2	4.9 ± .1	5.4 ± .2
Hair Development[b]	4.8 ± .3	4.8 ± .2	4.5 ± .3	4.8 ± .2
Incisor Eruption[b]	12.7 ± .4	12.1 ± .2	12.2 ± .4	11.7 ± .3
Eyelid Opening[b]	16.9 ± .2	16.6 ± .3	16.8 ± .2	17.3 ± .3
Surface Righting[b]	14.6 ± .4	14.3 ± .4	14.3 ± .6	14.0 ± .4

[a] percent of total number of pups born

[b] values are the litter means ± SE in days

( ) number of litters in each dose group

Table 4
Pup Total Body, Liver and Brain Growth

	Day		
	1	7	14	21

Dose	Body Weight (gm)			
Control	6.1 ± .2(24)	11.9 ± .4	28.4 ± .8	48.4 ± 1.4
20 PPM	6.0 ± .2(12)	12.7 ± .4	29.5 ± 1.1	49.9 ± 1.9
50 PPM	6.4 ± .2 (6)	11.4 ± .5	26.5 ± .7	45.2 ± 1.4
75 PPM	6.1 ± .2 (6)	11.8 ± .3	26.4 ± .6	46.4 ± 1.4

Liver Weight mg/gm Body Weight

Control	52.8 ± 2.8	31.8 ± 0.8	30.0 ± 1.4	39.0 ± 1.2
20 PPM	56.3 ± 5.8	29.7 ± 1.1	28.9 ± 1.7	36.7 ± 1.7
50 PPM	53.0 ± 2.4	31.0 ± 1.4	28.9 ± 0.9	34.7 ± 1.1
75 PPM	59.1 ± 0.7	29.9 ± 2.6	29.6 ± 1.1	37.1 ± 1.5

Brain Weight mg/gm Body Weight

Control	37.8 ± 2.9	46.0 ± 1.4	40.5 ± 3.4	29.6 ± 3.1
20 PPM	39.7 ± 2.3	45.0 ± 1.7	38.6 ± 0.9	28.9 ± 3.6
50 PPM	35.0 ± 1.6	56.4 ± 1.8	44.6 ± 0.7	32.0 ± 0.5
75 PPM	38.0 ± 2.4	46.1 ± 2.2	41.9 ± 0.6	29.7 ± 0.5

Values are the mean of litter weights ± SE

( ) = Number of Litters in each dose group

Table 5

Accumulation of Liver Protein, DNA and Cholesterol in Control and

Hydrogen Sulfide Exposed Rat Pups and Dams

		Day			
	1	7	14	21	DAMS
Dose	Protein mg/gm of tissue				
Control	270 ± 14(24)	292 ± 13	228 ± 3	211 ± 11	232 ± 12(24)
20	258 ± 36(12)	302 ± 6	230 ± 5	219 ± 8	230 ± 12(12)
50	260 ± 55 (6)	288 ± 11	236 ± 8	208 ± 8	237 ± 13 (6)
75	264 ± 41 (6)	298 ± 14	231 ± 7	211 ± 10	258 ± 14 (6)

DNA mg/gm of Tissue

Control	2.7 ± .2	3.9 ± .4	3.7 ± .2	2.6 ± .1	1.3 ± .2
20 PPM	2.4 ± .1	4.1 ± .1	4.4 ± .1	2.6 ± .1	1.3 ± .1
50 PPM	2.3 ± .1	4.8 ± .6	2.4 ± .3	2.0 ± .1	1.3 ± .1
75 PPM	2.7 ± .2	3.9 ± .4	3.6 ± .1	2.7 ± .1	1.7 ± .2

Cholesterol mg/gm of Tissue

Control	3.0 ± .6	4.4 ± .2	4.2 ± .1	4.4 ± .3	2.7 ± .1
20 PPM	2.5 ± .3	4.5 ± .1	4.1 ± .1	4.0 ± .2	2.7 ± .1
50 PPM	2.7 ± .4	3.9 ± .2	4.3 ± .1	4.3 ± .2	2.6 ± .1
75 PPM	2.6 ± .6	4.3 ± .2	4.4 ± .1	4.4 ± .5	3.0 ± .1[*]

Values are the mean litter weights ± SE

( ) = Number of Litters per dose

[*] significantly different from control ($p \leq .05$)

Table 6

Accumulation of Brain Protein, DNA and Cholesterol in Control and

Hydrogen Sulfide Exposed Rat Pups and Dams

Dose	1	7	14	21	Dam
		Protein mg/gm of Tissue			
Control	214 ± 14	204 ± 9	164 ± 18	157 ± 2	157 ± 4(24)
20 PPM	205 ± 6	189 ± 12	165 ± 4	156 ± 3	151 ± 6(12)
50 PPM	223 ± 16	209 ± 10	179 ± 25	172 ± 26	159 ± 7 (6)
75 PPM	226 ± 14	205 ± 12	162 ± 20	154 ± 5	169 ± 4 (6)

DNA mg/gm of Tissue

Dose	1	7	14	21	Dam
Control	3.0 ± .1	1.6 ± .1	1.8 ± .1	2.3 ± .1	1.3 ± .1
20 PPM	2.9 ± .1	1.6 ± .1	1.6 ± .1	2.2 ± .1	1.5 ± .1
50 PPM	2.6 ± .1	1.6 ± .1	1.5 ± .1	1.7 ± .1	1.2 ± .1
75 PPM	3.1 ± .1	1.7 ± .1	1.9 ± .1	1.8 ± .1	1.4 ± .1

Cholesterol mg/gm of Tissue

Dose	1	7	14	21	Dam
Control	3.1 ± .3	3.6 ± .1	5.5 ± .6	6.4 ± .5	16.3 ± .3
20 PPM	3.3 ± .4	3.6 ± .2	6.1 ± .2	7.4 ± .2	15.3 ± .6
50 PPM	3.1 ± .3	3.4 ± .2	5.1 ± .3	6.3 ± .1	15.6 ± .5
75 PPM	3.5 ± .9	3.8 ± .3	6.2 ± .4	7.2 ± .8	17.5 ± .8

Values are the mean Litter weights ± SE

( ) = Number of Litters Per Dose

Parturition Time Following H₂S Exposure
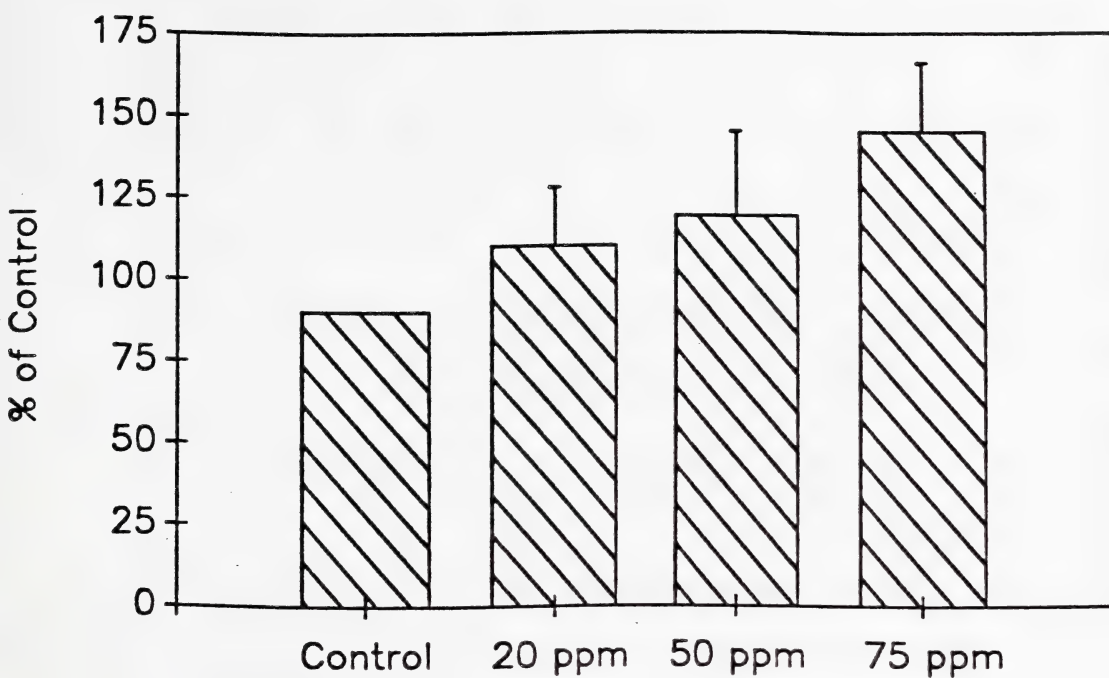
EXPOSURE TO LOW LEVELS OF HYDROGEN SULFIDE ELEVATES

CIRCULATING GLUCOSE IN MATERNAL RATS

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ABSTRACT

Although the lethal effect of hydrogen sulfide (H_2S) has long been known, the results of exposure to low levels of H_2S have not been well documented. Rat dams and pups were exposed to low levels of H_2S ($\leq$ 75 PPM) from day one of gestation until day 21 postpartum and analyzed for changes in circulating enzymatic activity and metabolites. Blood glucose was significantly elevated in maternal blood on day 21 postpartum at all exposure levels. This increase in glucose was accompanied by a possible decrease in serum triglyceride in the pups and in the dams on day 21 postpartum. There was no evidence of alterations in serum alkaline phosphatase, lactate dehydrogenase or serum glutamate oxalacetate transaminase.

INTRODUCTION

Hydrogen sulfide (H_2S) at high levels is a known lethal compound which inhibits cytochrome oxidase and other metal containing biological compounds by binding to metal ions. H_2S or the metabolic products of H_2S can also disrupt critical disulfide bonds in essential cellular proteins possibly causing rapid neurological dysfunction, respiratory paralysis and with time, a disruption of cellular metabolism (Beauchamp et al., 1984). Hydrogen sulfide has been reported to cause specie and tissue specific changes in alkaline phosphatase, acid phosphatase, adenosine triphosphate, succinate dehydrogenase, an accumulative reduction of cerebral cytochrome oxidase, alterations in bile flow and reduction in cerebral protein synthesis after acute exposure (Beauchamp

et al., 1984; Elovoara et al., 1978). Other cellular constituents were also altered with a reduction in brain RNA, glutathione (Beauchamp et al., 1984) and a specie specific reduction of brain lipids at low levels of H_2S (Gay et al., 1968). In the rabbit, 72 PPM H_2S (30 min/ day for 5 days) increased serum phosphate while lowering alkaline phosphatase activity, serum copper, iron, and α1-globulin levels (Smilkstein et al., 1985). Acute exposure to high levels of H_2S have been reported to cause hyperglycemia, secondary to adrenal stimulation, in several animals (Zburzhinskii, 1958) including humans (Levine, 1986). Whether this hyperglycemic response is a long term effect has not been investigated but may be important in view of the report that α-ketocarboxylic acids reduced the lethality of H_2S (Hume and Dulaney). Almost all of these responses have been noted with acute high H_2S concentration exposures, however, little information on the effects of subchronic or chronic exposures in the adult or in the developing animal have been described. This report is part of a study to determine whether low levels of H_2S ($\leq$ 75 PPM) alter maternal, fetal or neonatal growth and development using the rat model. The early fetal and neonatal growth period is a period which is highly susceptible to toxic compounds and any physiological alteration at this time could have long-term consequences on growth, development and metabolism (Buelke-Sam and Kimmol, 1979). The development of various enzymatic activities and metabolite levels during growth of the rat have been well documented (Kaplan, 1983) and can be employed to analyze for potential changes caused by H_2S exposure. This report documents the levels of serum glucose, triglycerides and cholesterol and enzymes during subchronic exposure to low levels of H_2S.

METHODS

After acclimating for one week, male and female Sprague-Dawley rats 80-90 days of age were allowed to cohabitate for a maximum of five days or until positive evidence of mating. Positive evidence of mating was the detection of sperm in the vaginal lavage and this occasion was designated day G1 of gestation. All animals were housed in plastic cages and maintained on a 12 hour light-dark cycle with ad libitum food and water supplies except during the seven hour per day period of exposure to H_2S. Exposure concentrations were 20 PPM, 50 PPM and 75 PPM.

Gestating dams were exposed to H_2S in an acrylic dome-shaped inhalation chamber fabricated from a modified design described by Laskin and Drew (1970). This chamber contained a 12-compartment circular galvanized cage and was approximately 90 liters in volume. The total animal volume did not exceed five percent of the total volume of the chamber. Exposed animals were continuously observed and chamber conditions were monitored for temperature, pressure, humidity and H_2S concentration. Hydrogen sulfide concentration was measured at the animal level with a pre-calibrated digital GFG model GMA-100 monitoring system (Gesellshaft für Gerätebau, Dortmund, West Germany) modified to measure a maximum concentration of 200 PPM.

Air supply to the chamber was room air drawn through a HEPA filter with a vacuum blower, mixed with certified H_2S (2000 PPM in nitrogen from Matheson Gas) with the maintenance of an oxygen concentration of

20-21% and an airflow of 20 chamber air changes per hour. The exposure mixture was passed through an orifice plate to measure flow rates, through a diffuser in the top of the chamber. A homogenous mixture of H_2S equilibrated at the exposure concentration within 10 minutes of the introduction of H_2S and was maintained within ±5% of the desired concentration at all times. The diffused gas passed through the chamber and out a double port at the bottom of the chamber. Vented H_2S and H_2O were trapped using a sodium hydroxide solution and dry ice traps before release into an exhaust duct.

Twenty gravid females were placed in two matched groups, and maintained under identical conditions except for exposure of the experimental group to H_2S at the designated concentration (20,50 or 75 PPM). Each group was culled to 6 litters with 12 pups in each litter at parturition (Day 1 PP). Serum samples were collected by exsanguination of pups on days 1,7,14 and 21 PP. Maternal blood samples for serum were collected from the inferior vena cava following pentabarbital anesthesia (35 mg/kg IP) on day 21 PP. Serum samples were stored at 4°C and analyzed within 48 hrs. Serum levels of protein (Doumas and Basil, 1975), glucose (Kosmider et al., 1966), triglycerides (Bucolo and David, 1973), cholesterol (Allain et al., 1974), lactate dehydrogenase (Gay et al., 1968), alkaline phosphatase (Laskin and Drew, 1970) and glutamate oxalacetate transaminase (Henry et al., 1960) were determined using an Instrumentation Laboratory Multistat III Analyzer.

Statistical analysis of the data was carried out using analysis of variance and Duncan's multiple range test or Student-Newman-Keuls Test. The mean values are the means for each litter exposed. A P value of $\leq$ 0.05 was considered significantly different.

RESULTS

Analysis of serum glucose revealed that exposure of pups to 20,50 or 75 PPM H_2S did not significantly alter the circulating levels of glucose. However, dams from these same litters had glucose levels which were approximately 50% greater in the animals at all exposure levels (Table 1). All the elevations in circulating glucose were significantly greater than the control values. Because of the increase in circulating glucose at 75 PPM, analyses of circulating triglycerides and cholesterol were carried out on the remaining 20 and 50 PPM exposures. Triglyceride and cholesterol levels were determined using serum collected at 10:00 hrs on each day of sampling, therefore stabilizing the prandial state of the mothers who consumed a meal when the dark cycle began at 19:00 hrs the evening before collection of the sample.

Triglyceride concentrations (Table 1) were reduced in the pups and dams in an apparent dose-dependent manner, but because of the large variation in litter means, these changes were not statistically significant except in dams at 50 PPM. At each time period, the levels of circulating triglyceride in pups were reduced by 20% at 50 PPM and by 25% in the dams at 21 days PP. Serum triglyceride and cholesterol

levels were approximately 3 fold higher in the pups than in maternal circulation. Those animals exposed to 50 PPM H_2S had an elevated cholesterol level on day 7 PP and 21 PP and in maternal blood on day 21 PP (Table 1). These values were not statistically different from the controls but suggested a trend which can be verified by the accumulation of a greater number of data points. Serum protein, LDH, SGOT and alkaline phosphatase activities were determined and as shown in Table 2, there were no differences between the mean serum activities from exposed animals and activities from the control animals.

DISCUSSION

Measured circulating enzymatic activities and protein did not change in the pups or dams exposed to H_2S at any concentrations tested. Although alkaline phosphatase activity was reportedly reduced in cerebellar cortex, lung and kidney at low levels in vivo (Beauchamp et al., 1984) and when blood alkaline phosphatase was exposed to extremely high levels of H_2S in vitro (Komberg and Horecker, 1955), in the present study neither blood, brain nor liver alkaline phosphatase activity was altered (Table 2). The discrepancies between these data and previous studies could be the result of differences in time of exposure and to the lower doses. Many of the studies reporting a reduction in alkaline phosphatase activity were short term exposures at both high and low concentrations of H_2S (Komberg and Horecker, 1955), while the present exposures were for 6 hours per day for 6 weeks. This longer period of exposure may allow the animal to adjust to early exposure with a return to normal activity levels with time. Since

neither serum LDH nor SGOT activities were altered in these animals, no tissue damage producing enzyme leakage was apparent as reported for rabbit heart (Komberg and Horecker, 1955) nor was there any direct inhibition of circulating enzyme activity in serum.

The elevation in maternal glucose after long-term exposure to low levels of H_2S could be the result of either a reduction in uptake and metabolism of glucose or the overproduction of glucose by adult hepatic tissue. The reason for the difference in glycemic response between adult and neonate rats was not apparent. The neonatal rat rapidly develops the ability to utilize glucose for lipid synthesis and has a high gluconeogenic and lipid oxidation capacity (Kaplan, 1983). During this neonatal development many tissues depend heavily on lipid metabolism as a source of energy and the glycemic response may occur only after the pups are weaned and begin to depend on a high carbohydrate diet. This perturbation of glucose homeostasis could be secondary to decrease in metabolism caused by an inhibition of tissue cytochrome oxidase (Haider et al., 1980; Beauchamp et al., 1984), removal of α-ketocarboxylic acids from metabolism (Hume and Dulaney, 1988) or destruction of insulin control of glucose metabolism by breakage of receptor or insulin disulfide bonds. The suppression of serum triglyceride levels also suggested that there was decreased use of carbohydrates for de novo synthesis of circulating triglycerides in the liver, the major site for synthesis of circulating triglycerides.

A previous low concentration 90 day exposure of adult male and female rats (Fisher 344 and Sprague-Dawley) and mice ($B_6C_6F_1$) to H_2S

did not demonstrate an alteration in blood glucose, proteins nor ketones (Chemical Industry Institute of Technology, 1983). The difference between the present study and the CIIT study was that female rats in this study were lactating animals with elevated oxytocin levels. Oxytocin is known to possess insulin-like activity controlling glucose metabolism and lipogenesis (Hanif et al., 1982) and initial evidence suggests that the oxytocin receptor was compromised in the presence of low levels of sodium sulfide, the nonvolatile salt of H_2S in uterine tissue (Franklin et al., 1989). Therefore, further studies will be required to determine if serum glucose levels are elevated because of disruption of insulin or oxytocin receptors or because of a perturbation of metabolism at a post-receptor site.

The long-term effects of elevated glucose levels have been a subject of discussion for some time (Kosmider and Zajusz, 1966). A growing body of evidence supports the hypothesis that chronically elevated glucose levels can produce retinopathy, neuropathy, nephropathy and increase severity of cardiac and peripheral atherosclerosis (Kosmider and Zajusz, 1966). The long-term effects of chronic or subchronic exposure to low levels of H_2S will have to be evaluated in light of this elevation in serum glucose.

Exposure of rats to low levels of hydrogen sulfide gave no evidence of tissue damage with leakage into serum of alkaline phosphatase, LDH or SGOT. Blood glucose was elevated in the adult female rat accompanied by a possible reduction in serum triglyceride in the pups and the maternal rat.

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Key Words: Hydrogen Sulfide, Rat, Hyperglycemia, Growth and
             Development

REFERENCES

Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W., and Fu, P. C. 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20:470-475.

Beauchamp, R. O., Bus, J. S., Popp, J. A., Coreiko, C. J. and Andjelkovich, D. A. 1984. A critical review of the literature on hydrogen sulfide toxicity. CRC Rev. Toxicol. 13(1):25-67.

Bucolo, G. and David, H. 1973. Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem. 19:476-482.

Buelke-Sam, J. and Kimmol, A. 1979. Development and standardization of screening methods for behavioral teratology. Toxicology 20:17-30.

Chemical Industry Institute of Techology (CIIT) 1983. 90 day vapor inhalation toxicity study of hydrogen sulfide in B_6C_3F mice, Fisher 344 rats and Sprague-Dawley rats.

Doumas and Basil, T. 1975. Standards for total serum total protein

assays; A collaborative study. Clin. Chem. 21:1159-1166.

Elovoara, E., Tossavainen, A. and Savalaivan, H. 1978. Effects of subclinical hydrogen sulfide intoxication on mouse brain protein metabolism. Exper. Neurol. 62:93-98.

Franklin, K. J., Hayden, L. J., Roth, S. H. and Moore, G. J. 1989. Inhibition of oxytocin induced contraction following low dose exposure to sodium sulfate. Proc. Can. Fed. Biol. Soc. 32:111.

Gay, R. J., McComb, R. B. and Bowers, G. N., Jr. 1968. Optimum reaction conditions for human lactate dehydrogenase isoenzymes as they affect total lactate dehydrogenase activity. Clin. Chem. 14:740-753.

Haider, S. S., Hasan, M. and Islam, F. 1980. Effects of air pollutant hydrogen sulfide on the levels of total lipids, phospholipids and cholesterol in different regions of the guinea pig brain. Ind. J. Exp. Biol. 18:418-424.

Hanif, K., Goren, H. J., Hollenberg, M. D. and Lederis, K. 1982. Oxytocin action: Mechanisms for insulin-like activity in isolated rat adipocytes. Molec. Pharmacol. 22:381-388.

Henry, R. J., Chamon, M., Golub, O. J. and Beckman, S. 1960. Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase and lactic acid dehydrogenase. Am. J. Clin. Path. 34:381-398.

Hume, A. S. and Dulaney, M. D., Jr. 1988. The effectiveness of various α-ketocarboxylic acids in preventing sulfide-induced lethality. Toxocologists 8:28.

Kaplan, S. H. 1983. Ontogenesis of hormone action: insulin, adrenal

corticoids, thyroid hormones, and growth hormone. In The Biological Basis of Reproductive and Developmental Medicine, ed. J. B. Warshaw, pp. 289-300. Elsevier: New York.

Komberg, A. and Horecker, B. L. 1955. Glucose-6-Phosphate Dehydrogenase. In Methods in Enzymology, eds. Colowich and Kaplan, Vol. 1, pp. 323-327. New York: Academic Press.

Kosmider, S., Rogala, E., and Pacholek, A. 1966. Studies on the toxic action mechanism of hydrogen sulfide. Int. Arch. Arbeitsmed. 22(1):60-66.

Kosmider, S. and Zajusz, K. 1966. Some enzyme and mineral balance disturbances in cerebellum during experimental subacute hydrogen sulfide poisoning. Zentralbl. Allg. Pathol. Anat. 109(4):411-418.

Laskin, S. and Drew, R. T. 1970. An inexpensive portable inhalation chamber. Ann. of Indust. Hyg. 31:645-646.

Levine, R. 1986. Monosaccharides in Health and Disease. In Annual Reviews of Nutrition, ed. R. E. Olson, Vol. 6, pp. 211-224: Annual Reviews Inc., Palo Alto, Calif.

McComb, R. B. and Bowers, G. N., Jr. 1972. Study of optimum buffer conditions for measuring alkaline phosphatase activity in human serum. Clin. Chem. 18:97-104.

Smilkstein, M. J., Bronstein, A. C., Pickett, H. M. and Rumack, B. H. 1985. Hyperbaric oxygen therapy for severe hydrogen sulfide poisoning. J. Emerg. Med. 3:27-30.

Zburzhinskii, V. K. 1958. The effect of hydrogen sulfide on the blood sugar level. Pharmacol. Toxicol. 21:411-415.

Table 1

Pup and Maternal Levels of Glucose, Triglyceride and Cholesterol Following
Exposure to Hydrogen Sulfide

| Dose | Days | | | Maternal |
	7	14	21	
		Glucose (mmoles/L)		
Control(24)	3.9 ± .4	6.1 ± .4	6.9 ± .3	5.2 ± .8
20 PPM(12)	3.7 ± .6	5.9 ± .5	7.0 ± .3	7.1 ± .7*
50 PPM(6)	3.4 ± .5(6)	6.0 ± .4	6.9 ± .4	7.5 ± .5*
75 PPM(6)	4.0 ± .4(6)	6.5 ± .3	7.1 ± .3	7.8 ± .7*
		Triglyceride (mg/L)		
Control(18)	134 ± 19	208 ± 11	212 ± 2	77 ± 6
20 PPM(12)	131 ± 12	186 ± 15	230 ± 24	65 ± 5
50 PPM(6)	108 ± 6	169 ± 16	170 ± 19	58 ± 5*
		Cholesterol (mg/dl)		
Control(17)	118 ± 6	136 ± 7	160 ± 10	44 ± 4
20 PPM(12)	109 ± 5	137 ± 5	151 ± 17	34 ± 3
50 PPM(6)	118 ± 9	154 ± 11	201 ± 19	50 ± 8

values are the means of litter values ± SE
( ) = number of litters analyzed
* means which are significantly different from controls with P≤0.05

Table 2

Pup and Maternal Serum Enzymatic Activity & Protein Levels From Animals Exposed to Varying Amounts of Hydrogen Sulfide[a]

Alkaline Phosphatase IU/Liter

Dose	Days 7	14	21	Maternal
Control	471 ± 36(17)	553 ± 17 (19)	534 ± 20 (18)	429 ± 38 (18)
20 PPM	490 ± 38(12)	564 ± 16 (12)	575 ± 18 (11)	348 ± 58 (12)
50 PPM	488 ± 27(6)	524 ± 14 (6)	465 ± 17 (6)	500 ± 39 (5)
75 PPM	464 ± 43(6)	572 ± 54 (6)	560 ± 60 (6)	429 ± 77 (6)

Lactate Dehydrogenase IU/Liter

Dose	7	14	21	Maternal
Control	966 ± 29	1223 ± 58	1187 ± 47	1052 ± 147
20 PPM	1036 ± 46	1245 ± 49	1104 ± 95	946 ± 85
50 PPM	1088 ± 54	1254 ± 53	1118 ± 106	990 ± 120
75 PPM	1010 ± 36	1210 ± 64	1220 ± 110	1100 ± 111

Serum Glutamic Oxaloacetic Transaminase IU/Liter

Dose	7	14	21	Maternal
Control	118 ± 17	152 ± 9	146 ± 11	108 ± 12
20 PPM	116 ± 15	145 ± 12	131 ± 16	97 ± 8
50 PPM	119 ± 14	153 ± 15	148 ± 18	110 ± 11
75 PPM	120 ± 18	142 ± 13	138 ± 12	105 ± 9

Serum Protein g/dl

Dose	7	14	21	Maternal
Control	3.7 ± .1	4.8 ± .1	5.7 ± .1	6.6 ± .1
20 PPM	3.4 ± .1	4.5 ± .1	5.3 ± .1	6.3 ± .1
50 PPM	3.6 ± .1	4.5 ± .1	5.7 ± .1	6.2 ± .2
75 PPM	3.4 ± .1	4.6 ± .1	5.7 ± .1	6.6 ± .2

[a] data from pups are the means of litter values ± SE in international units
( ) = number of litters analyzed

The Neurophysiological Effects of Low Concentrations of
Hydrogen Sulphide on the Developing and Mature Nervous Systems

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Abstract

It is well established that the environmental pollutant, hydrogen sulphide (H_2S), can be extremely toxic to both animals and humans at high concentrations; however, there is little information regarding the effects of long term exposure to low levels. H_2S is a major component of sour gas and a by-product of more than 70 industrial operations. It has been referred to as a broad spectrum toxicant that can elicit a wide variety of biological responses. Prolonged exposure to low concentrations may be a form of "chronic poisoning". Numerous neurophysiological symptoms have been associated with long term exposure to low levels, such as nervousness, poor memory, headache, sleep disturbances, insomnia, drowsiness, fatigue, weakness of extremities, disturbed equilibrium, vertigo, convulsions, anxiety, agitation, and delirium. Exposure to high concentrations can produce unconsciousness, coma, paralysis of respiratory and cardiovascular centres leading to death. These effects strongly suggest the central nervous system (CNS) is the major target organ for H_2S toxicity. Studies in our laboratory using in vitro neuronal model preparations have confirmed that low concentrations of H_2S can produce both a concentration and time dependent alteration of neuronal function. Developing and/or immature organisms are particularly susceptible to the adverse effects of most toxicants. We have studied the effects of low concentrations of H_2S on the developing organism and have observed that the dominant effects of H_2S appear to occur in the developing CNS. Exposure to H_2S produced a significant increase in the density of

Purkinje cells and decrease in the granule/Purkinje cell ratio in the cerebellum of the developing rat. There was also a significant alteration in both the dendritic architecture and overall growth process. Levels of putative amino acid neurotransmitters (asparate, GABA, glutamate, taurine but not glycine) were altered in the exposed developing rat brain. Although the effects of low concentrations of H_2S on neuronal tissue may be subtle, the results of our studies may explain the many behavioural responses reported and suggest that structural and functional abnormalities may occur during development.

INTRODUCTION

Hydrogen sulphide (H_2S) is a colourless gas that is both an environmental and industrial pollutant (Beauchamp et al, 1984). Acute exposure to high concentrations is highly toxic and often fatal. H_2S has a characteristic offensive odour of "rotten eggs" that can be detected by the olfactory system at concentrations as low as 0.2 ppm. The gas is heavier than air, soluble in both aqueous and organic solvents, and at physiologic pH, approximately one-third of H_2S exists in the undissociated form (H_2S) and two-thirds as the dissociated (HS^-) ion (Beauchamp et al, 1984).

H_2S is one of the leading causes of sudden death in the workplace (Ellenhorn & Barceloux, 1988). It is a major component of natural gas deposits such as "sour gas" and a variety of environmental natural sources resulting from bacterial decomposition of vegetable and animal proteinoceous material. High, often lethal concentrations are contained in manure tanks, slurry pits and sewers. H_2S is a by-product of more than 70 industrial processes including petrochemical refineries and plants, pulp and paper mills, viscose rayon production, sulphur extraction, iron smelters, heavy water production, and tanneries (Beauchamp et al, 1984). The known toxicity of H_2S on both animal and human subjects has been recently reviewed (Alberta Health, 1988; Beauchamp et al, 1984). It has been established that concentrations greater than 700 ppm are rapidly fatal to humans, and levels lower than 50 ppm can produce many adverse effects (Beauchamp et al, 1984). Most organ systems are susceptible to the effects of H_2S, and thus this agent has been referred to as a

broad spectrum toxicant (Alberta Health, 1988). The biological responses are varied, and the susceptibility or threshold responsiveness to the effects of H_2S are different, perhaps a function of dose, time or rate of exposure (Evans, 1967). The effects of prolonged exposure to low concentrations are not well established, and it has been proposed that the toxicity may be cumulative (Savolainen, 1980; Warenycia et al, 1987). A recent publication concluded that "there remains a lack of good scientific data about the long-term, low level chronic effects on community population and on the work force..." (Alberta Health, 1988).

Effects on the Nervous System

The neurophysiological effects of acute, high dose H_2S intoxicant have been reviewed (Alberta Health, 1988; Beauchamp et al, 1984), and well documented (see Table 1). Although sensory, motor and autonomic nervous pathways are involved, the primary target organ appears to be the central nervous system (CNS) (Amman, 1986; Ellenhorn and Barceloux, 1988). The CNS is complex, both in structure and function and extremely vulnerable to the effects of many toxicants, yet there is a rather considerable "functional reserve" that may account for a "subtle" effect resulting from an initial insult. Depression of the CNS can occur at 200 ppm H_2S (Beauchamp et al, 1984). The primary cause of death in acute high dose poisoning has been attributed to the paralysis of respiration resulting from depression of central respiratory centres (Beauchamp et al, 1984; Smith and Gosselin,

1979). It is likely that most of the systemic effects of chronic low dose H_2S intoxication (see Table 2), as well as the clinical symptoms following recovery from non-lethal accidental poisoning (see Table 3) are a result of the direct interactions with various CNS centres (Amman, 1986). At very low concentrations, H_2S is a mild general depressant. Early studies in animals have reported that chronic exposure to low levels (7 ppm) produced altered cortical dendrites in rats (Duan, 1959). Sandage (1961) described brain abscesses in mice exposed to 20 ppm for 90 days; in contrast to the results of a recent CIIT report (1983) indicating no abnormal behaviour or pathology in mice and rats exposed to low levels of H_2S for 90 days. Higuchi and Fukamachi (1977) reported that rats exposed to 200 ppm H_2S exhibited a reversible inhibition of avoidance response to behavior.

There are many factors which may influence the sensitivity of the CNS to the toxic effects of H_2S. Some of the most relevant include interactions with drugs (eg. central depressants, ethanol, anesthetics etc.) exposure to other chemicals (short chain alcohols and hydrocarbons), disease and/or pathological conditions and age (Alberta Health, 1988).

The Developing Nervous System

The developing organism is continuously in the process of rapid growth and maturation. In humans, the brain develops structurally both in utero and during the first few months of the postnatal period. Following birth, the mind develops (Bloom and Lazerson, 1988), that is, billions of cells are organized and

connected to each other to form a functional system. Development, per se, is not without error (Cowan, 1979), however, there is considerable evidence that the developing CNS is particularly susceptible to the effects of various toxicants (Galli, Manzo and Spencer, 1988). The immature, or indeed, the developing CNS, may respond to toxic compounds in a very different manner than the mature or adult system. Given that the developing or immature organism lacks a number of defense mechanisms such as metabolic processes and a blood brain barrier, there is potential for greater risk or injury due to chemical insult (Galli et al, 1988).

The development of the brain consists of several well-defined steps (see Table 4). Each step may be altered by the effects of a toxicant such as H_2S, and yield to premature or enhanced cell death. The sequence of cell proliferation is unique for each region, and elimination of excess neurons (programmed cell death) is characteristic of functional or behavioural maturity. The maximum rate of development, for example in the neocortex, is approximately 250,000 cells per minute (Cowan, 1979). The fully developed (mature) brain consists of approximately 100 billion neurons and 10^{15} synapses. Circuit formation of the CNS is not limited to the period in utero; formation of most synapses in the cortex occur after birth, although no new cells are added. Postnatal development of the nervous system is affected by interactions of the organism with the physical, intellectual, emotional and social environment; experience affects the processing of sensory and cognitive inputs. Genetic specification determines the size, shape, transmitter and basic blueprint or

patterning of a neuron, and hormones and other factors can alter development (Cowan, 1979).

There is a lack of information regarding the effects and severity of toxicity of H_2S to the developing and maturing organism. The greater potential for H_2S to produce toxic effects on the developing and maturing CNS has been the focus of research in our laboratories for the past several years, and has provided the basis of the studies described in this report.

The Effects of H_2S on Neuronal Activity

There is considerable evidence that high dose H_2S produces adverse effects on nervous tissue (Beck, Cormier and Donini, 1979; Beck, Donini and Maneckjee, 1983). It was shown nearly 60 years ago (Schmitt and Beck, 1930) that sodium sulphide (the volatile salt of H_2S) could affect nerve conduction. More recent publications have demonstrated that H_2S can alter the compound action potential of amphibian sciatic nerves (Beck et al, 1983) and produce concentration-dependent biphasic effects on peripheral nerves (Beck et al, 1983). It was postulated that these effects may be due to a blockade of sodium currents (Warenycia et al, 1989), suggesting that mechanisms similar to anesthetics may be responsible for the loss of central neuronal function.

The crayfish stretch receptor (muscle receptor organ) has been used in our laboratory as an in vitro model system to study the effects of various drugs, chemicals and toxicants on neuronal activity and excitable membrane properties of a single sensory neuron (Roth, 1980; MacIver and Roth, 1987). Using extracellular

recording techniques the effects of sodium sulphide (Na_2S) and sodium bisulphide (NaHS), the volatile salts of H_2S and gaseous H_2S have been examined on the physiological (stretch) and chemically-induced firing activity of the neuron. The methods have been previously described (Tan and Roth, 1983; 1984). In brief, stretch receptors of the crayfish, Procambarus clarkii, were isolated and suspended in physiological solution in a glass tissue chamber. Glass suction electrodes were applied onto the dorsal nerve to record action potentials (Figure 1). All solutions were prepared using filtered water just prior to each experiment.

It was observed that the discharge activity of the sensory neuron was altered by Na_2S in a rather complex time-dependent manner. In order to simplify the analysis, three phases were identified. Phase A was associated with the concentration and time-dependent reduction of action potential amplitude (Figure 2); the amplitude returned to control levels within a few minutes. The frequency of discharge activity began to increase in the presence of Na_2S (Phase B) following Phase A and reached a maximum within approximately 5 minutes (Figure 3). The enhanced firing activity during Phase B progressed toward lower frequencies with time, but still remained well above control levels. The maximum discharge activity during Phase B was also concentration-dependent (Figure 4). The period just prior to wash with control physiological solution was termed Phase C, which did not appear to be as dependent on concentration as Phase B.

Relative conduction velocities of action potentials along the dorsal neuron were determined using two suction electrodes spaced approximately 3-5 mm apart. The distance between peak heights of the two recorded wave forms in the presence of Na_2S were measured and compared to control values. As shown in Figure 5, the relative conduction velocities of treated neurons did not change significantly from controls at concentrations of Na_2S that altered discharge activity.

The effects of NaHS were essentially similar to those of Na_2S, except that during Phase A, the amplitude of the action potential was not reduced; rather, the discharge activity or firing rate was depressed in a concentration-dependent manner. Experiments using solutions bubbled with H_2S were also conducted, and the results were similar to those with Na_2S. The effective concentrations of gaseous H_2S solutions were determined using a sulphide (Orion) electrode. The differences observed between the two salts, Na_2S and NaHS, have not yet been explained. All the effects of the sulphide salts were reversible at the lower concentrations ($<10^{-4}M$), but were often irreversible at concentrations greater than 100 μM. The complex effects of H_2S on the sensory neuron are similar to those previously observed (Tan and Roth, 1983; 1984) and strongly suggest that H_2S can alter electrical membrane properties. The decrease of action potential amplitude could likely be due to a transient blockade of sodium channels, an increase in membrane resistance or hyperpolarization of the neuron (Edwards, Terzuolo and Washizu, 1963; Klie and Wilhoner, 1973). A mechanism of action similar to anesthetic

agents (Roth 1980) which has been suggested as a mechanism for H_2S (Beck et al, 1979; 1983) is also likely. The changes of firing activity may be related to a combination of actions on membrane resting potential, generator potential, membrane resistance, activation and/or inhibition of electrogenic pump activity or ionic channel kinetics. It is feasible that multiple sites and mechanisms are involved, which still remain to be be elucidated.

Alteration of CNS Development

The known effects of hydrogen sulphide toxicity in the central nervous system emphasize the high dose acute effects in the adult. For example, Lund and Wieland (1966) reported necrosis in the cerebral cortex and reduction in cerebellar Purkinje cells of rhesus monkeys exposed to hydrogen sulphide (500 ppm) for 20 mins. The effects of prolonged exposure to low levels have not yet been established, and there is little scientific information on the effects of subchronic exposure either on the adult or developing organism. Only one previous study appears to have examined the effects of H_2S on development; Glebova (1950) reported that breast-fed infants of mothers working in artificial silk factories exhibited retarded development and listlessness. Studies in our laboratory have utilized the rat as a test animal to examine the effects of low dose H_2S on the developing mammalian CNS.

Time-pregnant Sprague-Dawley rats were exposed to H_2S (75 ppm or less) for 7 hr per day from day 5 postcoitus until day 21

postnatal in a custom designed plexiglass environmental chamber. Control animals were exposed in an identical manner without H_2S. The 90 litre chamber contained a 12 compartment stainless-steel cage and total animal displacement did not exceed 5% of total volume. H_2S concentration was continuously monitored with a digital GFG (model GMA-100) system. A mixture of H_2S and room air was passed through an orifice plate to measure flow rate sufficient to produce complete volume changes every 3 minutes. Random selected pups (8 exposed and 8 control) were euthanized at sample times of 7, 14 and 21 days postnatal. Rats were decapitated, brains rapidly removed and specific regions dissected and processed as previously documented (Hannah, Roth and Spira, 1988; Hannah, Hayden and Roth, 1989).

Effects of H_2S on the Morphological Development of Central Neurons

The population densities of Purkinje and granule cells in the cerebellum were quantitated on days 7 and 14 postnatal. Exposure to 75 ppm H_2S resulted in a 20% increase in the density of surviving Purkinje cells as measured along the primary fissure (Figure 6), whereas there was no significant change in the mean number of granule cells (Figure 7). The observation that H_2S can produce morphological changes in the cerebellum suggests that many other neuronal populations may be at risk.

Alteration of Amino Acid Content in Developing CNS

Various amino acids have been identified as putative neurotransmitters in the developing rat cerebellum (Cutler and Dudzinski, 1974). Alterations in the levels of these amino acids during development may produce significant changes to the growth and maturation processes. The levels of aspartate, GABA, glutamate, glycine and taurine were quantitatively measured using high-performance liquid chromatography (Hannah, Hayden and Roth, 1989). With the exception of glycine, the levels of aspartate, glutamate and GABA were significantly reduced below control levels by day 21 postnatal. Glutamate levels were unchanged at day 7 and 14, but reduced at day 21 (Figure 8). Thus, exposure to H_2S produced alterations in four of five amino acids tested. The observed alterations during this critical phase of development may have chronically affected the activity of the neurotransmitters, their receptor sensitivity or their individual target areas. The precise consequences of these changes are as yet unknown but are likely to result in long term behavioral and/or motor problems.

Alterations in Architecture and Growth Processes of Developing Cerebellar Purkinje Cells

Architectural development and overall growth process of the cerebellar Purkinje cell dendrites can be quantitatively analyzed using vertex analysis. This form of computer analysis is a third-generation program developed by Berry and Flinn (1984). It

differs from earlier topographical methods such as network analysis (Berry et al, 1975; Berry and Bradley, 1976) by reducing networks to a small number of vertex types. Structures, such as Purkinje cell dendrites, which grow by a pattern of lengthening and branching can be mathematically analyzed as tree structures (Hannah et al,1988). The analysis of branch point type, frequency and distribution in the tree plus values such as branch length and number of generations of branches can be used to determine whether growth is proceeding randomly or non-randomly; random growth suggests that growth is occurring without external influence.

Vertex analysis of the treated Purkinje cells at day 21 postnatal revealed several significant alterations in both the dendritic architecture and overall growth process. Exposure to either 20 or 50 ppm H_2S extended the dendritic processes of Purkinje cells significantly prior to branching compared to control preparations (Figure 9). In addition, treated cells showed a significantly greater number of branches within the middle of the dendritic field with significantly fewer branches at the periphery where growth should be occurring (Figure 10). In terms of growth characteristics, a comparison of branch type ratios demonstrated that the treated cells were significantly less random in their growth compared to controls.

Conclusions

The physiochemical properties of hydrogen sulphide are consistent with the observed effects on neuronal tissue. The

lipophilic nature of the gas predicts access to all tissues, and is a common property of many agents that are able to alter the excitability of neuronal cells (Roth, 1980). Studies on model systems such as the isolated sensory neuron will be useful to elucidate mechanisms of action and to test potential antagonists and/or prophylactic measures for H_2S poisoning.

The complexity of the CNS in both structure and function often yield results that are difficult to interpret. The observed H_2S-induced decrease in GABA content cannot simply be explained as a loss of Purkinje cells, but may be related to either decreased production or increased utilization. The reduced levels of the other amino acids in treated animals may be due to a cellular loss, alteration in synthesis or decrease in utilization. It is likely that most of these substances, with the possible exception of GABA, are present in many cell types and may also possess non-transmitter related roles.

It must be concluded, however, that the effects of low dose, chronic exposure to H_2S may result in perturbation of the normal growth process of the developing CNS ultimately leading to abnormal function. Many of the results emphasize the "subtle" nature of the toxicity of low levels of H_2S.

Future studies are directed towards the long-term effects, reversibility, plasticity and behavioural outcomes of chronic exposure during development.

Acknowledgements

FIGURE LEGENDS

Figure 1   Schematic diagram of an isolated sensory neuron
           suspended   between   forceps   (F)   and   a   microtension
           transducer (T) in a tissue chamber continuously perfused
           with physiological solution.   Glass suction electrodes
           (E_1, E_2) recorded extracellular action potentials.

Figure 2   Oscillograph showing the time-dependent depression of
           the action potential amplitude in the presence of 80 µM
           Na_2S.

Figure 3   Frequency histogram showing the time-dependent triphasic
           response of discharge activity in the presence of 50 µM
           Na_2S.

Figure 4   Concentration response curves of discharge activity in
           the presence of Na_2S during Phases B and C.

Figure 5   Relative conduction velocities (time between peak
           heights of action potentials of E_1 and E_2) did not
           change significantly during the three phases in the
           presence of Na_2S.

Figure 6   Mean number of Purkinje cells along the primary fissure
           of cerebellum at 7 and 14 days postnatal exposed to 75
           ppm H_2S compared to controls.

Figure 7   Mean number of granule cells along the primary fissure of cerebellum at 7 and 14 days postnatal exposed to 75 ppm H_2S compared to controls.

Figure 8   Effect of hydrogen sulphide (75 ppm) exposure on amino acid levels in the rat cerebellum. Relative amounts are expressed as a percent of control.

Figure 9   Mean vertex path length (mean distance between branches at equivalent order of branch point) of cerebellar Purkinje cells dendritics in the treated group at day 21 postnatal were increased compared to control.

Figure 10 The mean number of branches at equivalent order of cerebellar Purkinje cell dendrites was symmetrically different for the treated group compared to control.

TABLE 1. EFFECTS OF ACUTE H_2S SYSTEMIC INTOXICATION

sudden fatigue

dizziness

intense anxiety

convulsions

loss of olfactory function

unconsciousness

collapse

respiratory arrest

cardiac failure

death

TABLE 2.   EFFECTS OF CHRONIC H_2S INTOXICATION (<100 PPM)

lethargy

dizziness

loss of appetite

fatigue

headache

mental depression

irritability

poor memory

abnormal peripheral reflexes

GI disturbances

TABLE 3. SYMPTOMS FOLLOWING RECOVERY OF H_2S POISONING

neurasthesia

fatigue

nausea

anxiety

depression

peripheral acoustic neuritis

headache

insomnia

lack of initiative

irritability

poor or loss of memory

decreased libido

nystagmus

disturbed equilibrium

TABLE 4. DEVELOPMENT OF THE BRAIN

INDUCTION	induction of neural plate
PROLIFERATION	localized proliferation of cells in different regions
MIGRATION	migration of cells from region of generation
AGGREGATION	aggregation of cells to form identifiable regions
DIFFERENTIATION	cellular differentiation of immature neurons
CIRCUIT FORMATION	formation of connections with other neurons
PROGRAMMED CELL DEATH	selective death of certain cells
SYNAPSE REFINEMENT	elimination of some connections and stabilization of other connections

REFERENCES

Alberta Community and Occupational Health (1988) Report on H_2S
Toxicity, 65 pp.

Amman, H.J. (1986) A new look at physiologic respiratory response
to H_2S poisoning. J. Hazard Mat. 13: 369-374.

Beauchamp, R.O., Bus, J.S., Popp, J.A. et al. (1984) A critical
review of the literature on hydrogen sulfide toxicity. CRC Review
of Toxicology. 13: 25-97.

Beck, J.F., Cormier, F. and Donini, J.C. (1979) The combined
toxicity of ethanol and hydrogen sulfide. Toxicol. Letters 3:
311-313.

Beck, J.F., Donini, J.C. and Maneckjee, A. (1983) The influence of
sulfide and cyanide on axonal function. Toxicology. 26: 37-45.

Berry, M. and Bradley, P.M. (1976) The application of network
analysis to the study of branching patterns of large dendritic
fields. Brain Res. 109: 111-132.

Berry, M. and Flinn, R.M. (1984) Vertex analysis of Purkinje cell
dendritic trees in the cerebellum of the rat. Proc. R. Soc. Lond.
B. 221: 321-348.

Berry, M., Hollingworth, T., Anderson, E.M. and Flinn, R.M. (1975)
Application of network analysis to the study of branching patterns
of dendritic fields. In: G.W. Kreutzberg (Ed): Advances in
Neurology. Raven Press, New York, pp. 217-245.

Bloom, F.E. and Lazerson, A. (1988) Brain, Mind and Behavior.
W.H. Freeman and Co., New York, 394 pp.

Chemical Industry Institute of Toxicology (CIIT)(1983) 90 day
Vapor Inhalation Toxicity Study of Hydrogen Sulphide in B_6C_3F
Mice, Fisher 344 Rats andd Sprague-Dawley Rats.

Cowan, W.M. (1979) The development of the brain. Scientific Amer.
241: 112-133.

Cutler, R.W.P. and Dudzinski, D.S. (1974) Regional changes in
amino acid content in developing rat brain. J. Neurochem. 23:
1005-1009.

Duan, F.Z. (1959) Maximum permissible concentrations of hydrogen
sulfide in the atmosphere. Gig. Sanit. 24 (10) 12-17.

Edwards, C., Terzuolo, C.A. and Wishizu, Y. (1963) The effect of
changes of the ionic environment upon an isolated crustacean
sensory neuron. J. Neurophysiol. 26: 948-957.

Ellenhorn, M.J. and Barceloux, D.G. (1988) Medical Toxicology.
Elsevier Science Publishing Co. 1512 pp.

Evans, C.L. (1967) The toxicity of hydrogen sulphide and other
sulphides. Quart. J. Exp. Physiol. 52: 231-248.

Galli, C.L., Manzo, L. and Spencer, P.S. (Ed)(1988) Recent
advances in Nervous System Toxicology. Plenum Press, New York.
391 pp.

Glebova, K. (1950) Permissible tolerance of hydrogen sulphide in
the atmosphere. Gig. Sanit. 4: 19-21.

Hannah, R.S., Hayden, L.J. and Roth, S.H. (1989) Hydrogen sulfide
exposure alters the amino acid content in developing rat CNS.
Neuroscience Letters. 99: 323-327.

Hannah, R.S., Roth, S.H. and Spira, A.W. (1988) Effect of phenobarbital on Purkinje cell growth patterns in the rat cerebellum. Exper. Neurol. 100: 354-364.

Higuchi, Y. and Fukamachi, M. (1977) Behavioral studies on toxicity of hydrogen sulfide by means of conditioned avoidance responses in rats. Folia Pharmacol. Japan. 73 (3) 307 - 320.

Klie, J.W. and Wilhoner, H.H. (1973) Voltage clamp studies on the stretch response in the neuron of the slowly adapting crayfish stretch receptor. Pflugers Arch. 342: 93-104.

Kombian, S.B., Warenycia, M.W., Mele, F.G. and Reiffenstein, R.J. (1988) Effects of acute intoxication with hydrogen sulfide on central amino acid transmitter systems. Neurotoxicol. 9: 587-596.

Lund, O.E. and Wieland, H. (1966) Pathological-anatomical findings in experimental hydrogen sulfide poisoning (H_2S) - a study on rhesus monkeys. Int. Arch. Gewerhepathol. Bewerbehyg. 22: 46-54.

MacIver, M.B. and Roth, S.H. (1987) Anesthetics produce differential actions on the discharge activity of a single neuron. Europ. J. Pharmacol. 139: 43-52.

Roth, S.H. (1980) Molecular and cellular actions of anesthetic agents. Fed. Proceedings. 39: 1595-1599.

Sandage, C. (1961) Tolerance criteria for continuous inhalation exposure to toxic material II. Effects on animals of 90-day exposure to H_2S, methyl mercaptan, indole, and skatole. Armed. Ser. Tech. Infor. Agency (ASTIA AD 187 797).

Savolainen, H., Tenhunen, R., Elovaara, E. and Tossavainen, A. (1980) Cumulative biochemical effects of repeated subclinical hydrogen sulfide intoxication in mouse brain. Arch. Occup. Environ. Health. 46: 87-92.

Schmitt, F.O. and Beck, L.V. (1930) The effect of carbon monoside and of hydrogen sulphide on nerve irritability. Biol. Bull. 59: 269-274.

Smith, R.P. and Gosselin, R.E. (1979) Hydrogen sulfide poisoning. J. Occup. Med. 21: 93-97.

Tan, K-S and Roth, S.H. (1983) Comparative effects of extracellular ions on the discharge firing activity of an isolated neuron.   IRCS Med. Sci. 11: 296-297.

Tan, K-S and Roth, S.H. (1984) An in vitro study of copper and copper chelators on the crayfish stretch receptor. Neuropharmacol. 23: 683-689.

Warenycia, M.W., Goodwin, L.R., Francom, D., Reiffenstein, R.J., Dieken, F.P. and Taylor, J.D. (1987) REgional brain distribution of sulfide after H_2S poisoning. Proc. 24th Intern. Meeting of the International Association of Forensic Toxicologists. G.R. Jones and P.O. Singer (Eds) University of Alberta Press, Alberta.

Warenycia, M.W., Steele, J.A., Karpinski, E. and Reiffenstein, R.J. (1989) Hydrogen sulfide in combination with taurine or cysteic acid reversibly abolishes sodium currents in neuroblastoma cells. Neurotoxicology. 10: 203 - 209.
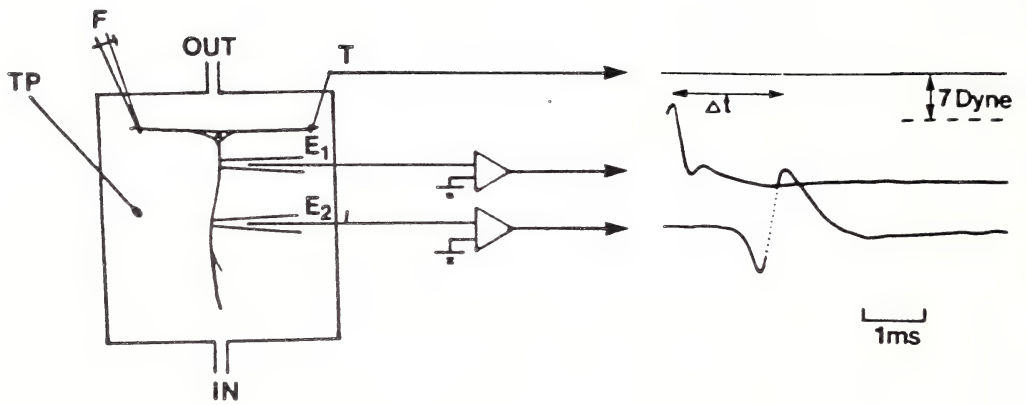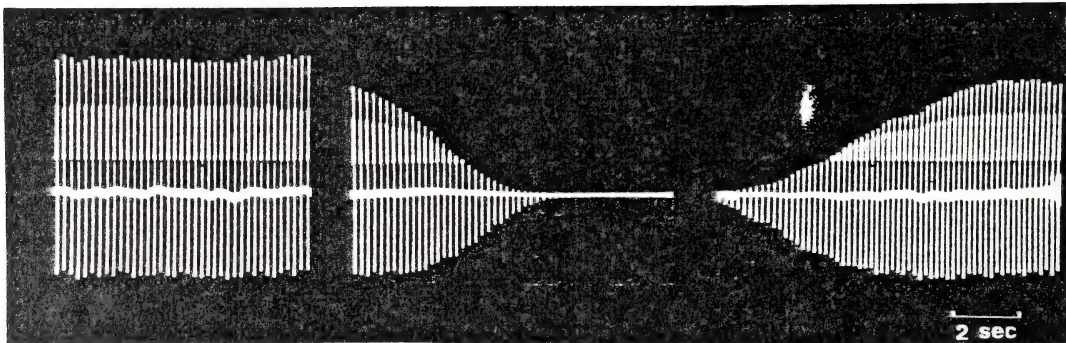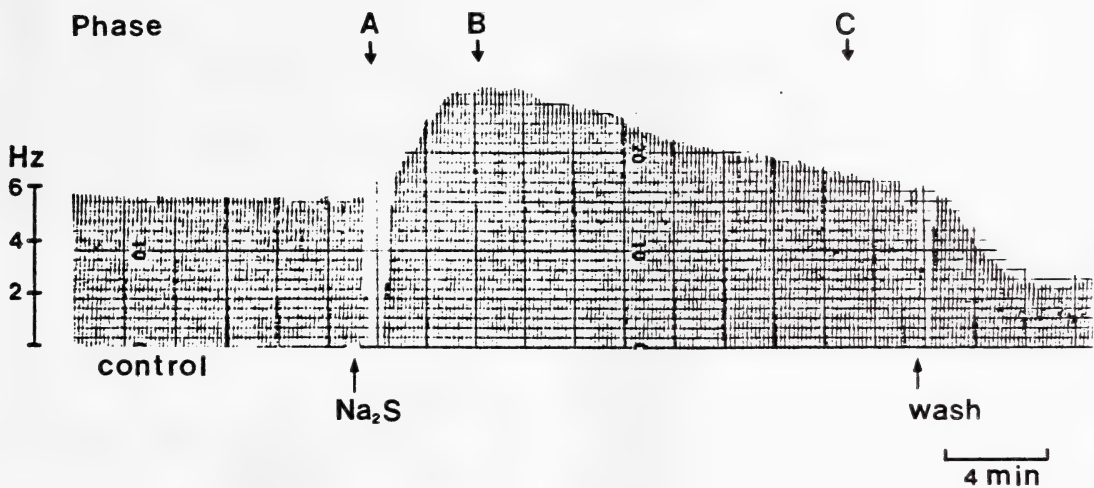
Figure 1

Figure 2
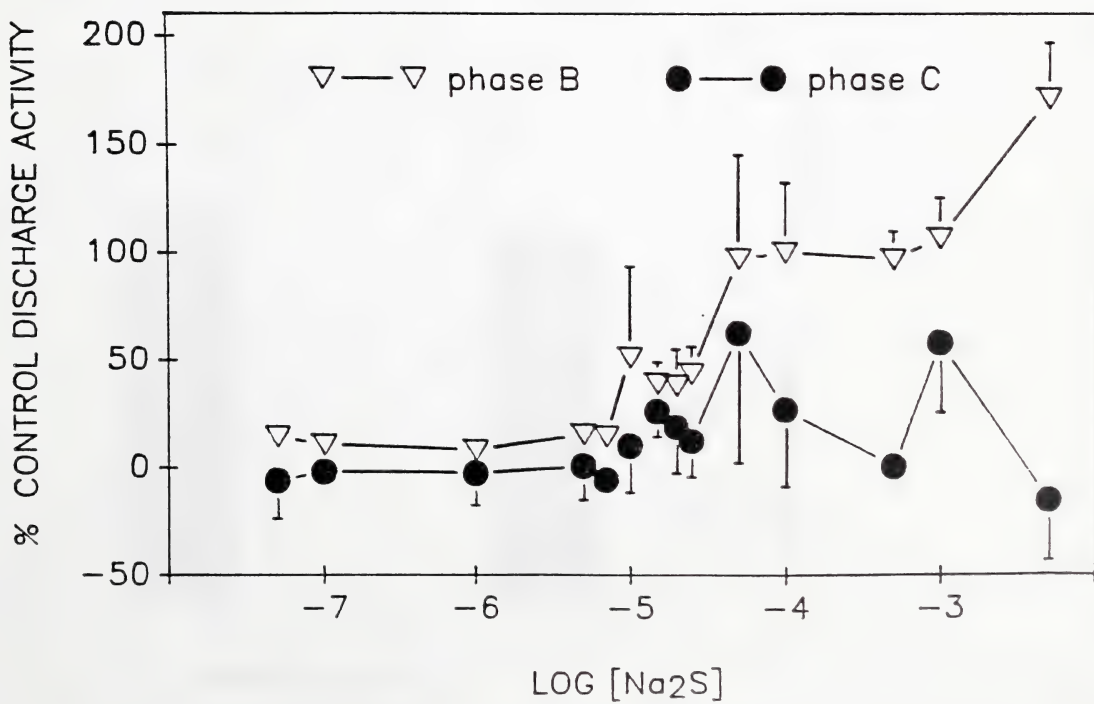
Phase

A B   C

Figure 3.

Figure 4

Effect of Na2S on MRO conduction
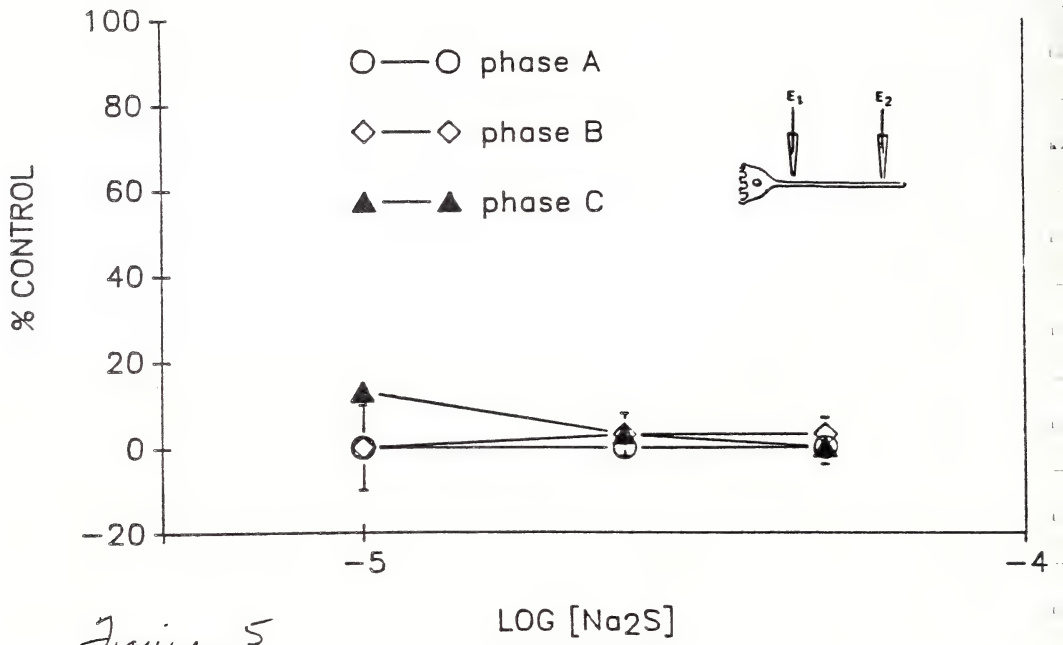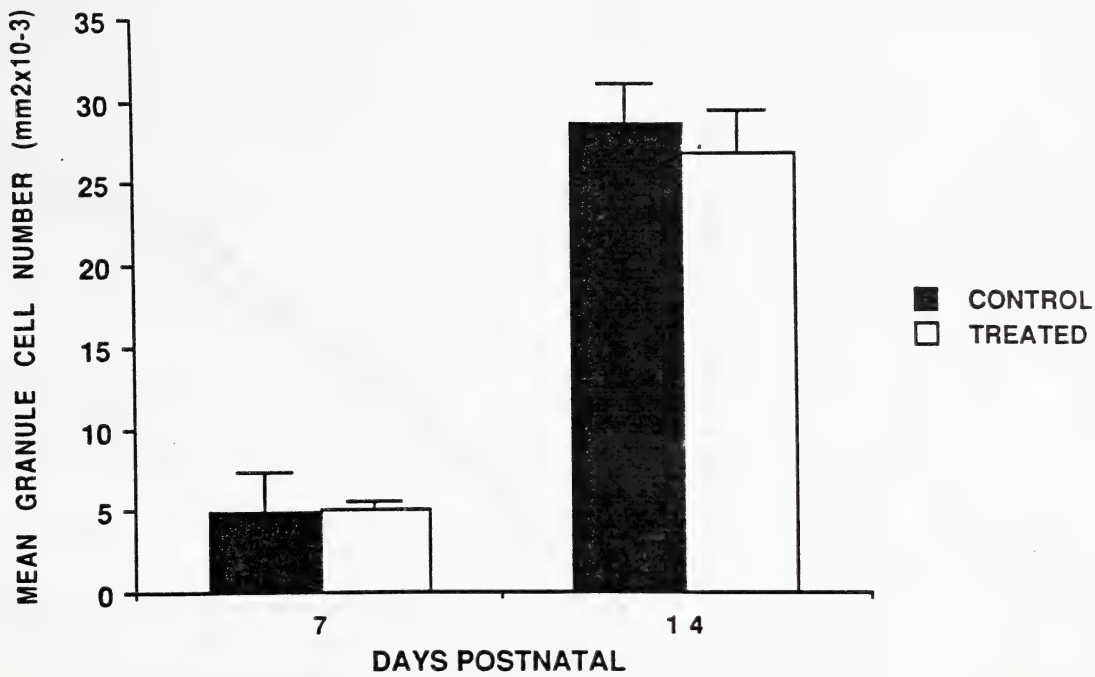
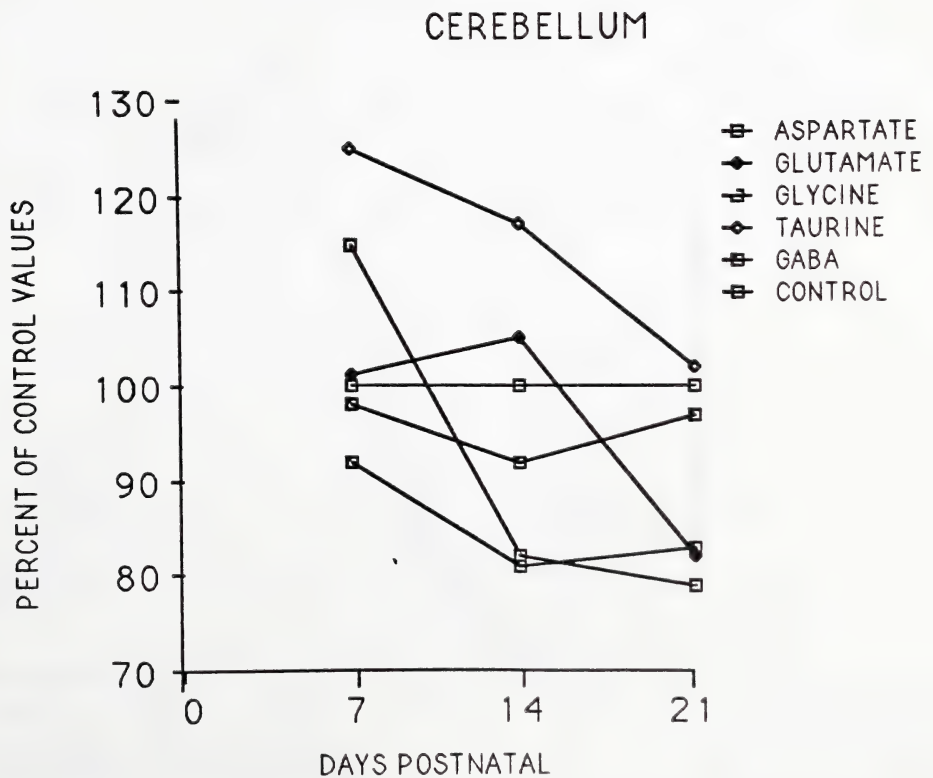

Figure 5

Figure 6

Figure 7
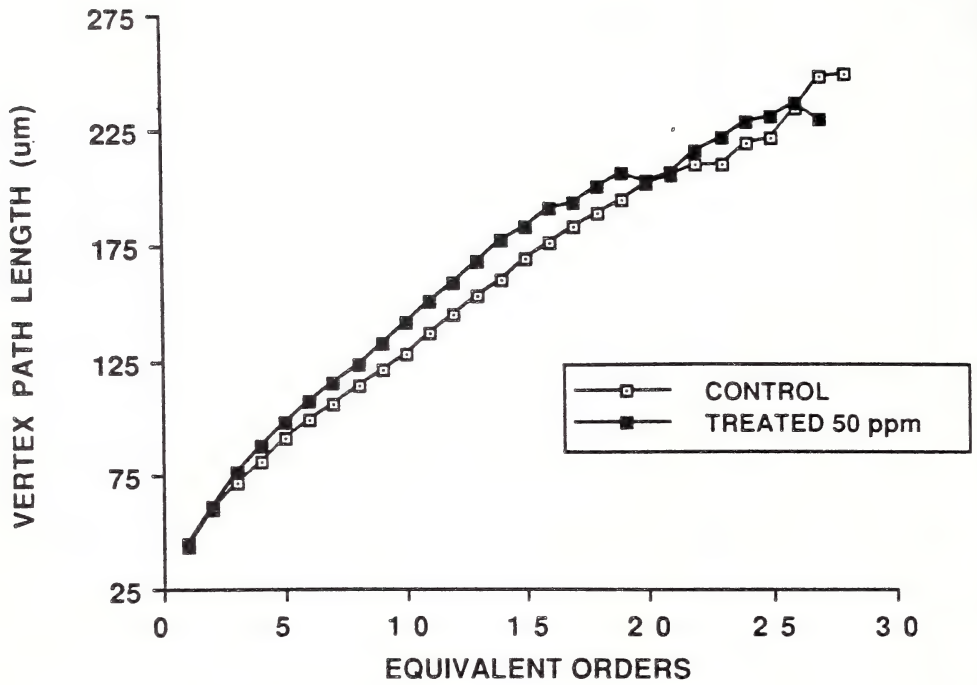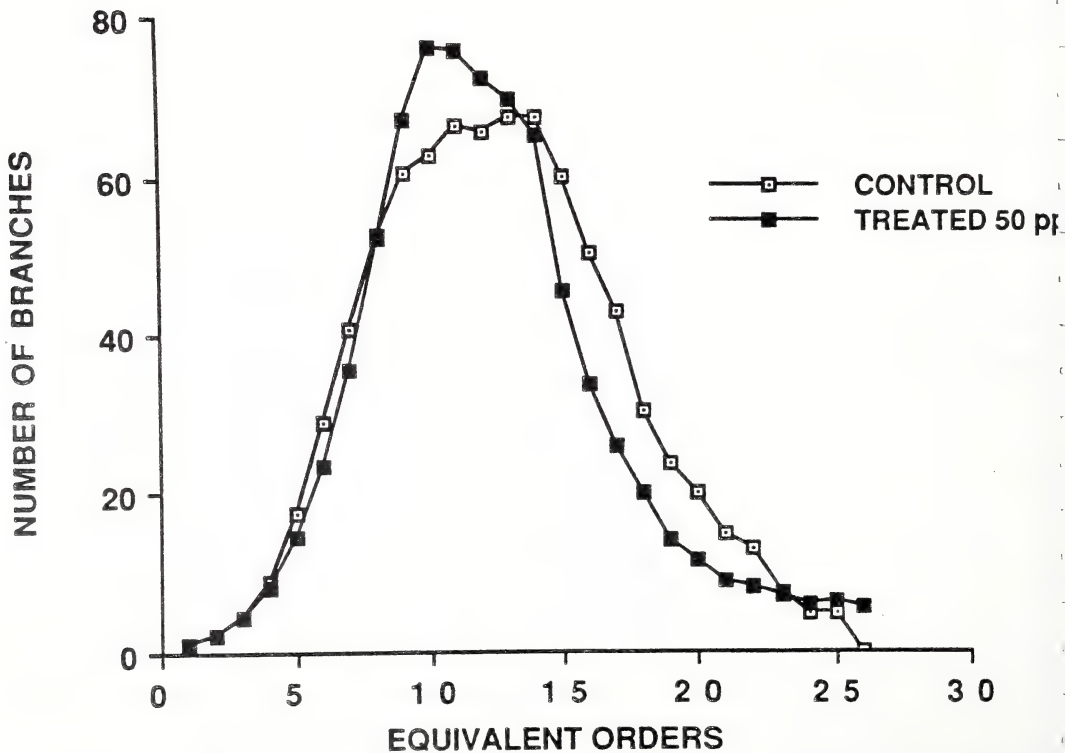
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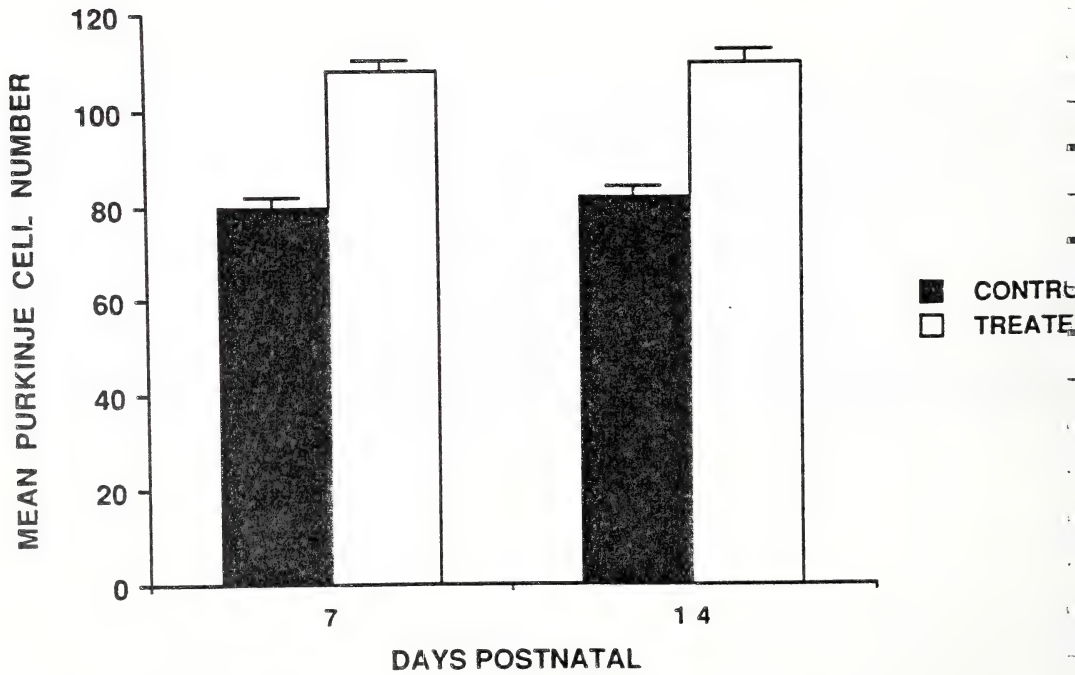
Figure 8

VERTEX PATH LENGTH

Figure 9

Hydrogen Sulphide in Biochemical Systems

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Abstract

Hydrogen sulphide is a natural product of the metabolism of sulphur containing amino acid compounds in tissue and by bacteria in the gastrointestinal tract. Hydrogen sulphide from these and exogenous sources can be detoxified by oxidation and excretion as sulphate, or by methylation in the intestinal mucosa and liver. Hydrogen sulphide and its oxidation products have been shown to react with disulphide and metal containing biochemical compounds which may serve a detoxication role, or if these compounds serve an essential function, this interaction could produce physiological changes. Hydrogen sulphide is rapidly absorbed into the blood stream where it is bound to protein and quickly oxidized when transported to the liver. At high concentration in vitro hydrogen sulphide inhibits mitochondrial oxidative metabolism and this is believed to be the mechanism of action for the lethal effects of hydrogen sulphide. Recent evidence indicates that hydrogen sulphide can also interact with plasma membrane receptor proteins causing a disruption of hormone signalling and possible alteration in energy metabolism and muscle contraction at low concentrations.

This review will discuss the possible interactions of hydrogen sulphide with biochemical compounds at various concentrations, with an analysis of possible chemical interactions and biological responses at anticipated circulating concentrations.

Key Words: Hydrogen sulphide, oxidation, disulphide, detoxification, toxicity

In high concentrations hydrogen sulphide (H_2S) is known to be a lethal compound. The mechanism of the lethal effect is believed to be the binding of hydrogen sulfide to metal ions complexed in biological compounds such as the ferric heme iron of cytochrome oxidase. The consequence of binding to heme iron in cytochrome oxidase is the inhibition of this terminal enzyme of electron transport and cessation of energy production followed by cell death if inhibition is not alleviated (Beauchamp et al., 1984). Recent studies (Beck et al., 1983) suggest a second mechanism of acute poisoning could be the result of interactions at the cellular plasma membrane level which causes rapid neurological dysfunction and respiratory paralysis, possibly as a result of disruption of critical disulphide bonds or disruption of membrane lipids.

Hydrogen sulphide has been reported to cause specie and tissue specific changes in alkaline phosphatase, acid phosphatase, adenosine triphosphatase, succinate dehydrogenase, an accumulative reduction of cerebral cytochrome oxidase, alterations in bile flow and reduction in cerebral protein synthesis after acute exposure (Beauchamp et al., 1984; Elovoara et al., 1978). Cellular constituents were also altered with a reduction in brain RNA, glutathione (Beauchamp et al., 1984) and a species specific reduction (Gay et al., 1968) of brain lipids at low levels of H_2S. In the rabbit 72 ppm H_2S (30 min/day for 5 days) increased serum phosphate while lowering alkaline phosphatase activity, serum copper, iron, and α1-globulin levels (Smilkstein et al., 1985). Acute exposure to high levels of H_2S have been reported to cause hyperglycemia, secondary to adrenal stimulation, in several animals

(Zburzhinskii, 1958) including humans (Levine, 1986). Whether this hyperglycemic response is a long term effect has not been investigated but may be important in view of the report that α-ketocarboxylic acids reduced the lethality of H_2S (Dulaney and Hume, 1988). The biochemical changes produced by H_2S have been reviewed previously and at this conference (Beauchamp et al., 1984; Khan, 1989).

In the time that I have available I would like to review what is known about the uptake, distribution and oxidation of H_2S and to outline the possible mechanisms of action for H_2S. H_2S is a normal metabolic product of the break down of biological materials containing sulphur. This presence of H_2S can be the result of anaerobic bacteria metabolism in the gastrointestinal tract or the result of the ingestion of fermentation products (Weisiger, 1980). The usual route of entry of H_2S into the body would be through the mucous membrane of the gastrointestinal tract where it can be metabolized or enter the portal blood supply to be rapidly distributed to various tissues and oxidized. However, toxic doses of H_2S very rarely are ingested but are inhaled acutely or chronically at low doses. Histochemical procedures (Voigt and Muller, 1955) have shown that inhaled H_2S is distributed to the brain, liver, kidney, pancreas and small intestine. Whole body radioautography (Curtis et al., 1972) and tissue sampling (Gunina, 1957) following administration of radiolabelled sodium sulphide intraperitoneally confirmed the wide distribution of ^{35}S-sulfide with an accumulation of radioisotope in lung. Intravenously administrated sodium sulphide is rapidly removed from the circulation with only 11% of the dose remaining in the blood plasma fraction after five minutes.

Approximately 14% of the label in blood was bound directly to protein as the persulphide and the remainder had been oxidized to sulphate (Curtis et al., 1972). Intraperitoneal or intravenous administration of ^{35}S-sodium sulfide was completely recovered as inorganic sulphate in urine or feces in 48 hours with only small amounts of other labelled compounds. However, after 48 hours only 71% of the orally administrated sodium sulphide was recovered as inorganic sulphate with the remainder believed to be lost as the volatile methanethiol or dimethylsulphide, the detoxification products of intestinal thiol-S-methyl transferase. Only trace amounts of ^{35}S-sodium sulphide were detected in expired air from animals given either intravenous or intraperitoneal doses of sodium sulphide (Beauchamp et al., 1984).

Physiological solutions of sulphide at equilibrium contain H_2S and the hydrosulphide ion (HS$^-$) with a distribution of approximately two thirds HS$^-$ and one third H_2S at pH 7.4. A solution in equilibrium with a 70 mg/m^3 (50 ppm) atmosphere of H_2S would contain a maximum concentration of approximately 4.8 μM total sulphide if one assumes no oxidation of the sulphide. H_2S is readily soluble in organic as well as aqueous solutions and can readily cross cellular plasma membranes to enter the circulation and tissue where it can again ionize to hydrosulphide (Beauchamp et al., 1984). The concentration of H_2S in membranes (a site of sensitive enzymes) is unknown.

Hydrogen sulphide metabolism proceeds by several pathways including oxidation, methylation, persulphide formation and binding to metal containing proteins. Oxidation to sulphate and excretion by the

kidney is the major pathway for the detoxification of H_2S as shown by isotopic studies of the fate of sulphide in vivo (Curtis et al., 1972; Gunina, 1957; Dziewiatkowski, 1945). In many tissues the initial step in the oxidation of the hydrosulphide ion takes place in the presence of O_2 and mitochondrial heme groups with the formation of free sulphur. The sulphur is believed to form a highly stable polysulphide intermediate (Sörbo, 1960; Baxter et al., 1958). Employing O_2, polysulphide is oxidized to thiosulphate using mitochondrial sulphide oxidase (Baxter and Van Reen, 1958a; Der-Garabedian, 1945). In mitochondria reduced glutathione (Frendo and Janik, 1967) stimulated the formation of sulphate from thiosulphate with a sulphite intermediate which was converted to sulphate by the action of sulphite oxidase (MacLeod et al., 1961). In vitro tissue perfusion studies demonstrated that liver and kidney, to a lesser extent, were the major sites of sulphide oxidation with a very small contribution to oxidation by the blood (Curtis et al., 1972). The thiosulphate intermediate of sulphide oxidation was also excreted by the kidney and a single study has shown it to be a potential indicator of exposure to hydrogen sulphide gas (Kangas and Savolainen, 1987). A second possible mechanism for the oxidation of H_2S is ferritin oxidation as suggested by Baxter and Van Reen (1985b). These studies demonstrate that isolated ferritin rapidly oxidized H_2S to sulphate and the high concentration of ferritin in the intestine may serve a detoxification role.

The second pathway for the detoxification of sulphide is by enzymatic methylation with thiol-S-methyl transferase as indicated

above. Methyl transferase activity was high in cecal and colonic mucosa with lesser activity in liver, lung and kidney. The elevated activity in mucosa and liver could provide detoxification of ingested and intestinal H_2S but the importance of this pathway has not been directly verified (Weisiger et al., 1980).

A third pathway for the interaction of H_2S with biochemical compounds is through the reaction with either metal or disulphide-containing proteins. These reactions with proteins can lead to either detoxification by binding of H_2S in an inactive form or toxic effects by destruction or inhibition of biological activity depending on the specific protein and H_2S concentration. Cytochrome oxidase, alkaline phosphatase, catalase, adenosine triphosphatase and methemoglobin are examples of metal containing proteins which interact with the hydrosulphide ion at high concentrations. Hydrosulphide binds to the ferric heme iron of isolated cytochrome oxidase, the enzyme activity is reversibly inhibited with a k_i of approximately 0.2 μM causing a loss of mitochondrial electron transport and ATP production (Nicholls and Kim, 1981). Binding to methemoglobin has been suggested as an effective antidote to hydrogen sulphide poisoning and the induction of methemoglobin with sodium nitrate has been shown to reduce the lethal effects of H_2S in mice (Smith and Gosselin, 1964). In a single study saturating amounts of H_2S inhibited blood alkaline phosphatase activity. This inhibition was reversed with the addition of $MgCl_2$ suggesting that the reduction in alkaline phosphatase activity documented in vivo may be the result of the reaction of H_2S with magnesium followed by precipitation and removal of magnesium sulphide

(Beauchamp et al., 1984). However, the direct inhibition of alkaline phosphatase at lower concentrations of H_2S (< 10 μM) has not been documented.

One of the interesting aspects of the presence of hydrogen sulphide in isolated particulate cytochrome oxidase preparations and in at least one mitochondrial study is the increased consumption of O_2 with increasing concentrations of H_2S. Using a beef heart mitochondrial preparation, Nicholls and Kim (1981) demonstrated that H_2S could serve as a substrate for cytochrome oxidase at high enzyme concentrations. Vetter, at this meeting, has also reported that oxygen consumption of liver mitochondria from sulphide tolerant fish can be stimulated by the addition of H_2S up to 20 μM total sulphide followed by inhibition of O_2 consumption at higher concentration (Vetter, 1989). Thus, there may be a biphasic response of cytochrome oxidase to H_2S with oxidation of H_2S at low concentrations followed by inhibition of the enzyme at higher concentrations.

There is growing evidence that one of the mechanisms for H_2S interaction with proteins is by reductive cleavage of protein and amino acid disulphide bonds producing persulphide and free sulphhydryl groups. This reaction has both toxic and therapeutic potential. Many essential enzymes, proteins, hormones and receptors contain essential disulphide bonds. If these essential bonds are reduced by H_2S (as shown with several other reducing compounds), the biological activity of these compounds will be compromised (Hanif et al., 1982). Bergstermann and Lummer (1947) suggested that the inhibition of

succinic acid dehydrogenase activity with H_2S is the result of the breakage of disulphide bonds (Smilkstein et al., 1985). More recently evidence has been presented that demonstrated that low levels (0.15 μM) of H_2S inhibited oxytocin-induced rat uterine contractions at the receptor (Franklin et al., 1989). This inhibition of oxytocin receptor function is similar to that produced by known disulphide reducing compounds (Hanif et al., 1982). Recent reports have also shown that the in vivo inhibition of monoamine oxidase can be reversed using dithiothreitol and reduced glutathione compounds known to be involved in disulphide cleavage and displacement of HS^- from proteins (Warenycia et al., 1989). The use of oxidized glutathione as an antidote for H_2S poisoning is based upon the reductive cleavage of the glutathione disulphide bond forming the nontoxic persulphide which can then slowly release H_2S for oxidation to sulphate (Smith and Abbanat, 1966). In this way oxidized glutathione (and also nonessential protein) serves as a detoxifying sink which then slowly releases the H_2S for oxidation to sulphate.

Other biochemical interactions

Recent studies by Dulaney and Hume (1988) have demonstrated an interaction between α-ketocarboxylic acids and H_2S. Pyruvate and α-ketoglutaric prophylacticly reduced the lethality of interperitoneal sodium sulphide in mice. The suggested mechanism of action for these compounds is a nucleophilic attack of hydrosulphide on the carbonyl group which results in scavenging and detoxification of H_2S.

Sulfhemoglobin formation _in_ _vitro_ is the product of a hydrogen peroxide-generated hydrosulphide free radical addition across a heme double bond. Although readily formed _in_ _vitro_ and a suggested product of exposure to H_2S in humans (Hoidal et al., 1986), very low levels of sulfhemoglobin were detected in humans or animals exposed acutely to H_2S (Curry and Gerkin, 1987).

Hydrogen sulphide, a normal product of metabolism, can be readily detoxified by oxidation, methylation or reaction with disulphide compounds or proteins. Depending on the concentration of H_2S in the tissue, the reaction of H_2S with either metal or disulphide containing proteins can produce a loss of essential protein function. There is a growing set of data which indicates that H_2S may interact with essential biochemical compounds and proteins causing disruption of biological function (Franklin et al., 1989; Warenycia et al., 1989; Dulaney and Hume, 1988). Continued analysis of these interactions will determine whether H_2S at low concentrations causes a toxic response.

References

ALLAIN, C.C., POON, L.S., CHAN, C.S.G., RICHMOND, W. and FU, P.C. 1974.
    Enzymatic determination of total serum cholesterol. Clin. Chem.
    20:470-475.

BAXTER, C.F., VAN REEN, R., PEARSON, P.B. and ROSENBERG, C. 1958.
    Sulfide oxidase in rat tissue. Biochim. Biophys. Acta.
    27:584-590.

BAXTER, C.F and VAN REEN, R. 1958a. Some aspects of sulfide oxidation
    by rat-liver preparations. Biochim. Biophys. Acta. 28:567-573.

BAXTER C.F. and VAN REEN, R. 1958b. The oxidation of sulfide to
    thiosulfate by metallo-protein complexes and by ferritin.
    Biochim. Biophys. Acta. 28:573-578.

BECK, J.F., DONINI, J.C. and MANECKJEE, A. 1983. The Influence of
    Sulfide and Cyanide on Axonal Function. Toxicol. 26:37-45.

BEAUCHAMP, R.O., BUS, J.S., POPP, J.A., COREIKO, C.J. and ANDJELKOVICH,
    D.A. 1984. A critical review of the literature on hydrogen
    sulfide toxicity. CRC Rev. Toxicol. 13:25-67.

BERGSTERMANN, H. and LUMMER, H.D. 1947. Action of hydrogen sulfide and
    its oxidation products on succinic acid dehydrogenase. Arch. Exp.
    Path. Pharmakol. 204:509-512.

CURRY, S.C. and GERKIN, R.D. 1987. A patient with sulfhemoglobin? Annals. of Emerg. Med. **16**:828-830.

CURTIS, C.G., BARTHOLOMEW, T.C., ROSE, F.A. and DODGSON, K.S. 1972. Detoxication of sodium ^{35}S-sulfide in the rat. Biochem. Pharm. 21:2313-2321.

DER-GARABEDIAN, M. 1945. The sulfide oxidase of higher vertebrates. Precipitation by alcohol. Comp. and Rend. Soc. Biol. **139**:310-316.

DULANEY, M., Jr. and HUME, A.S. 1988. Pyruvic acid protects against the lethality of sulfide. Res. Comm. Chem. Path. Pharmacol. 59:133-136.

DZIEWIATKOWSKI, D.D. 1945. Fate of ingested sulfide sulfur labeled with radioactive sulfur in the rat. J. Biol. Chem. **161**:723-729.

ELOVOARA, E., TOSSAVAINEN, A. and SAVOLAINEN, H. 1978. Effects of subclinical hydrogen sulfide intoxication on mouse brain protein metabolism. Exper. Neurol. **62**:93-98.

FRANKLIN, K.J., HAYDEN, L.J., ROTH, S.H. and MOORE, G.J. 1989. Inhibition of oxytocin-induced uterine contractions following low dose exposure to sodium sulfide. Proc. Can. Fed. Biol. Soc. 32:111.

FRENDO, A.K.J. and JANIK, Z. 1967. [^{35}S] Thiosulphate oxidation by rat

liver mitochondria in the presence of glutathione. Biochem. J. 103:791-795.

GUNINA, A.I. 1957. The metabolism of hydrogen sulfide (H_2S^{55}) injected subcutaneously. Bul. Exper. Biol. Med. 43:176-179.

HANIF, K., GOREN, H.J., HOLLENBERG, M.D. and LEDERIS, K. 1982. Oxytocin action: Mechanism for insulin-like activity in isolated rat adipocytes. Molec. Pharmacol. 22:381-388.

HOIDAL, C.R., HALL, A.H. and ROBINSON, M.D. 1986. Hydrogen sulfide poisoning from toxic inhalations of roofing asphalt fumes. Ann. Emerg. Med. 15:826-830.

KANGAS, J. and SAVOLAINEN, H. 1987. Urinary thiosulfate as an indicator of exposure to hydrogen sulphide vapour. Clinca. Chimica. Acta. 164:7-10.

KHAN, A.A. 1989. Biochemical effects of hydrogen sulfide toxicity. In: Proceedings of the International Conference on Hydrogen Sulfide Toxicity. Prior and Reiffenstein, eds. University of Alberta Press, Edmonton.

LEVINE, R. 1986. Monosaccharides in health and disease. In: Annual Reviews of Nutrition. R.E. Olson ed., Vol. 6, pages 211-224: Annual Reviews Inc., Palo Alta, Calif.

MacLEOD, R.M., FARKAS, W., FRIDOVICH, I. and HANDLER, P. 1961.
    Purification and properties of hepatic sulfite oxidase. J. Biol.
    Chem. **236**:1841-1846.

NICHOLLS, P. and KIM, J.K. 1981. Oxidation of sulphide by cytochrome
    aa_3. Biochim. Biophys. Acta. **637**:312-320.

SINITSAIN, S.N. 1962. Age of rats and sensitivity to hydrogen sulfide.
    Farmakol. **25**:232-240.

SMART, J.L. and DOBBING, J. 1971. Vulnerability of developing brain.
    II. Effects of early nutritional deprivation on reflex ontogeny
    and development of behaviour in rat. Brain Res. **28**:85-95.

SMILKSTEIN, M.J., BrONSTEIN, A.C., PICKETT, H.M. and RUMACK, B.H. 1985.
    Hyperbaric oxygen therapy for severe hydrogen sulfide poisoning.
    J. Emerg. Med. **3**:27-30.

SMITH, R.P. and ABBANAT, R.A. 1966. Protective effect of oxidized
    glutathione in acute sulfide poisoning. Toxicol. Appl. Pharmacol.
    9:209-212.

SMITH, R.P. and GOSSELIN, R.E. 1964. The influence of
    methemoglobinemia on the lethality of some toxic anions. II.
    Sulfide. Toxicol. Appl. Pharmacol. **6**:584-589.

SÖRBO, B. 1960. On the mechanism of sulfide oxidation in biological
    systems. Biochim. Biophys. Acta. **38**:349-351.

TUCHMANN-DUPLESSIS, H., HISS, D., MOTTOT, G. and ROSNER, I. 1975.
Effects of prenatal administration of acetylsalicylic acid in
rats. Toxicology 3:207-211.

VETTER, R.D. 1989. Hydrogen sulfide metabolism in marine organisms.
In: Proceeding of the International Conference on Hydrogen Sulfide
Toxicity. Prior and Reiffenstein, eds. University of Alberta
Press, Edmonton.

VOIGT, G.E. and MULLER, P. 1955. The histochemical effects of hydrogen
sulfide poisoning. Acta. Histochem. 1:223-226.

VORHEES, C.V., BUTCHER, R.E., BRUNER, R.L. and SOBOTKA, T.J. 1979.
Effects of prenatal administration of acetysalicylic acid in rats.
Toxicology 3:207-211.

VYTASEK, R. 1982. A sensitive fluorometric assay for the determination
of DNA. Anal. Biochem. 120:243-248.

WARENYCIA, M.W., FUNG, K. and REIFFENSETIN, R.J. 1989. Restoration of
monoamine oxidase (MAO) activity by dithiothreitol following
enzyme inhibition by NaHS. Proc. Can. Fed. Biol. Soc. 32:117.

WEISIGER, R.A., PINKUS, L.M. and JUKOBY, W.B. 1980. Thiol S-
methyltransferase: suggested role in detoxication of intestinal
hydrogen sulfide. Biochem. Pharm. 29:2885-2887.

WINICK, M. and NOBLE, A. 1965.   Quantitative changes in DNA, RNA and
    protein   during   prenatal   and   postnatal   growth   in   the   rat.
    Developmental Biology 12:451-466.

ZBURZHINSKII, V.K. 1958.   The effect of hydrogen sulfide on the blood
    sugar level.   Pharmacol. Toxicol. 21:411-415.

COMPLEX ACTIONS OF HYDROGEN SULFIDE AND ITS VOLATILE SALTS

ON THE DISCHARGE ACTIVITY OF THE CRAYFISH SENSORY NEURON

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Introduction

There is considerable evidence that hydrogen sulphide (H_2S) can produce adverse effects on nervous tissue (4,8,11). It is an environmental and industrial pollutant, highly toxic and often fatal at high concentrations. Chronic or repeated exposure to low levels of H_2S have been associated with a variety of psychological and neurophysiological reactions (1) such as nervousness, insomnia, fatigue, weakness of extremities, disturbed equilibrium, vertigo, poor memory, loss of appetite, anxiety, agitation, delirium and convulsions. Acute exposures have been related to signs of cerebral and extrapyramidal damage, facial paralysis, prolonged reaction times, absent or abnormal reflexes of both cranial and spinal levels, loss of short term memory, depression and epileptic like seizures (1). Depression of the nervous system can occur at 200 ppm, and rats exposed to this concentration exhibit a reversible inhibition of avoidance behavior (6). It is most likely that the systemic effects of H_2S on the central nervous system may result in paralysis of the respiratory center consequently leading to cardiac failure and death (1). Since the above effects are neural in origin, it is strongly suggested that the central nervous system is the major target organ for H_2S toxicity (1).

A recent publication demonstrated the short duration, anesthetic-like actions of H_2S (2); sulphide containing solutions altered the compound action potential of amphibian sciatic nerve. In addition, gaseous H_2S (at very high concentrations) was shown to produce a concentration dependent biphasic effect on peripheral nerves (2).

The purpose of the present study was to examine the effects of low concentrations of H_2S on the activity of an isolated neuron. The

results demonstrate that low concentrations of sodium sulphide and sodium bisulphide (volatile salts of H_2S) and gaseous H_2S bubbled into solution, all produced a time and concentration dependent alteration of the discharge activity of the isolated crayfish sensory neuron.

Methods

The isolated crayfish stretch receptor, a sensory neuron, has proven to be an excellent model to examine the effects of a variety of neural active substances (10). The methods used in the present study have been described previously (9,10,13,14). In brief, stretch receptor neurons of the crayfish, Procambarus clarkii were isolated and suspended in a small glass tissue chamber maintained at $10 \pm 1^{\circ}C$ and continuously perfused with modified Van Harreveld's solution [Van H] (15) at a constant flow rate of 10 ml/min. Glass suction electrodes were applied onto the dorsal nerve to record stretch induced action potentials. Discharge frequency histograms were generated with a microprocessor frequency counter and a hard copy was obtained on a chart recorder. Solutions of Na_2S and NaHS were prepared in Van H just prior to each experiment. All solutions were prepared using filtered water of approximately 18 Megohm resistivity (Barnstead RO Nanopure Water System).

Results

At a maintained stimulus (stretch), the discharge activity of the neuron plateaued to a rhythmical frequency of approximately 4-6 Hz. Solutions of Na_2S or NaHS at various concentrations were applied during this plateau phase.

Sodium sulphide Na_2S: The effects of Na_2S on the discharge activity were both time and concentration dependent.   In order to simplify the analysis of the time dependent effects, three phases were identified: Phase A occurred within approximately 1-2 min, Phase B about 5 mins and Phase C around 20 mins from time of administration.   Each phase was characterized by a different response.   Phase A initially was observed as a depressant effect on frequency; however, the amplitude of the extracellularly recorded action potential was also reduced in several experiments. The reduction of the amplitude appeared to be concentration dependent within a very narrow range approaching a quantal response. The amplitude began to return towards control levels within a few minutes with continued perfusion of Na_2S.   Following this initial response or phase, the frequency of firing began to increase and reached a maximum value in 5 mins.   This increase in frequency was termed Phase B and the maxium response was shown to be concentration dependent.

The enhanced firing activity progressed towards a lower frequency with time, but never reached control levels (Phase C).   Upon wash with fresh Van H, the neuron ceased to fire momentarily, then resumed firing with a slow decrease in frequency followed by a return to control level in approximately 30-40 min.   As shown in Figure 1, the maximum response of Phase B was concentration dependent, however, Phase C (period just prior to wash) did not appear to be as dependent on concentration as Phase B.   Conduction of velocities along the dorsal neuron were determined using two suction electrodes spaced approximately 3-5 mm apart.   The distance between the peak heights of the two action potentials were measured and the relative velocities in Na_2S solutions were compared to control.   It can be seen in Figure 2, that relative

conduction velocities were not significantly altered in the presence of Na_2S.

Sodium bisulphide (NaHS): The effects of NaHS were essentially similar to Na_2S except that during Phase A, the amplitude of the action potential was not reduced, rather the discharge frequency was depressed in a concentration dependent manner. Only three concentrations of NaHS were tested and the effects are shown in Figure 3. All three phases appeared to be concentration dependent for NaHS. Relative conduction velocities were not altered at concentrations up to $5x\ 10^{-5}$ M, but appeared to be slowed during Phase B at the higher concentrations studied (see Figure 4).

Solutions of gaseous H_2S were prepared by bubbling H_2S gas (2,000 ppm in nitrogen) into van H for approximately 5 mins. The resultant solutions were observed to have similar effects as Na_2S. Concentrations of H_2S solutions were measured using a sulphide electrode.

Relative conduction velocity determinations were not determined with the gaseous H_2S solutions.

All the effects at lower concentrations (ie. $< 10^{-4}M$) were reversible, however, effects at the higher concentrations ($>10^{-4}$) were often irreversible.

Discussion

It is apparent that low concentrations of H_2S as either the gas bubbled directly into solution or those produced by dissolving the volatile salts, Na_2S or NaHS, in Van H altered the firing activity of the sensory neuron in a characteristic manner. The response was rather complex; the effects were both time and concentration dependent. The threshold for the excitatory Phase B, was noted to be approximately 1 μM whereas a 10 fold increase in concentration was necessary for the depressant actions of Phase A. We have observed similar (11) time and concentration dependent actions of ions on this neuron which strongly suggests the effects of H_2S may be due to changes on electrical membrane properties.

The decrease in action potential amplitude could likely be due to a transient blockade of sodium channels resulting in an increase in membrane resistance or hyperpolarization of the neuron (5,7).Similar effects would also result from and enhanced activity of the Na-K ATPase pump. This is relatively unlikely for it has been shown that H_2S inactivates many enzyme systems such as the metalloproteins containing alkali metals (1). The role of calcium in the activity of the crayfish sensory neuron is well documented (3), therefore it possible that H_2S may interfer with either the channel or the intracellular concentration of calcium. H_2S is lipid soluble (1), a common physical property of most anesthetic agents (10), therefore it is possible that the mechanism(s) of action may be similar. This has been suggested previously (2).

Alteration of other ionic channels such as chloride, may also contribute to increased membrane resistance. At physiological pH, H_2S exists

primarily as the hydrosulfide anion (1), which may be very reactive with intracellular or membrane bound cations. It is interesting, that a recent study (16), reported no significant change in sodium currents or channel- kinetics of mouse neuroblastoma cells at concentrations of NaHS well above the maximum of the present study.

The enhanced firing of Phase B may be attributed to a decrease in membrane resistance leading to depolarization of membrane resting potential, or alteration of channel kinetics. It is also feasible that Na-K ATPase may be inhibited by H_2S thus altering resting membrane potential; we have observed in this laboratory that low concentrations of ouabain will enhance firing activity of the sensory neuron (unpublished data).
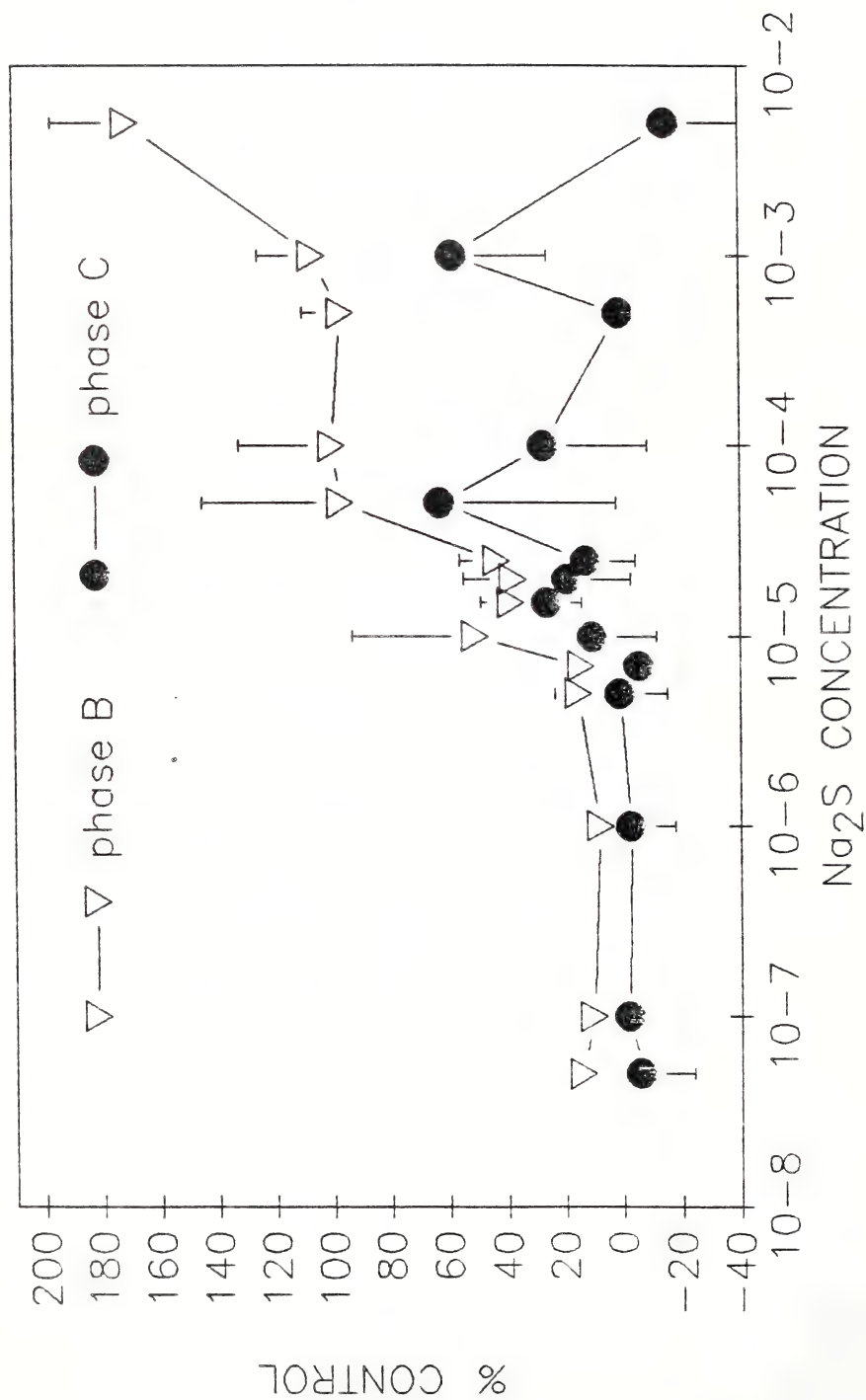
The time dependent effects may be related to the kinetics at the membrane level or within the microenvironment of the neuron. The ratio of fluid to cell volume is very high, and the flow rate is more than sufficient to overcome any loss of chemical either by evaporation or cell uptake. The changes of firing activity that occurred in the presence of the toxicant may be related to a combination of actions on membrane resting potential, generator potential, membrane resistance, activation and/or inhibition of electogenic pump activity or ionic channel kinetics. Although the results presented in this study do not explain the precise mechanisms for the neurotoxic effects of H_2S, considering the properties of H_2S, it is feasible that more than one site of action is involved and elucidation of these sites and mechanisms remain to be solved.
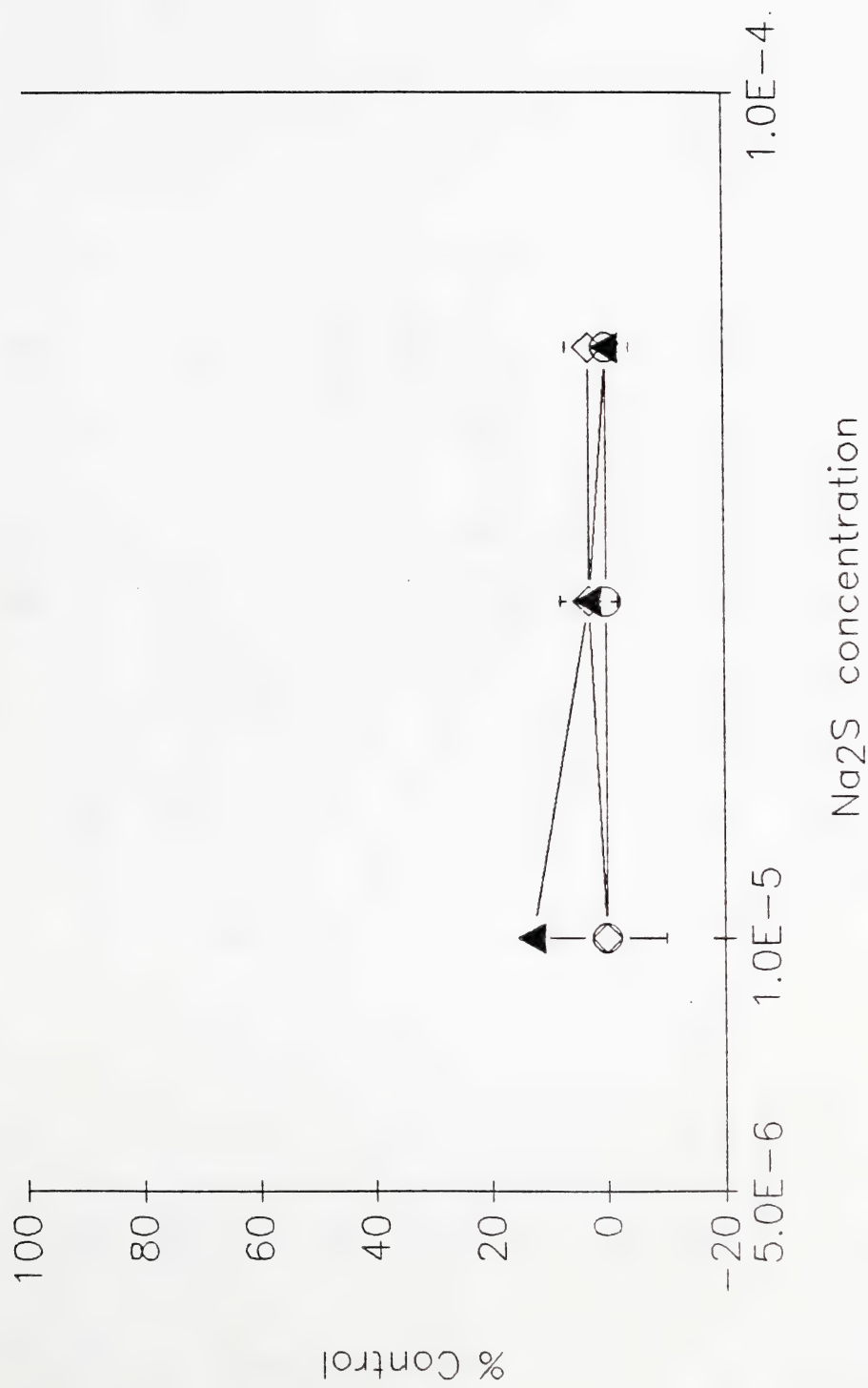
References

1. Beauchamp, R.O., Bus, J.S., Popp, J.A. et al. (1984) A critical review of the literature on hydorgen sulfide toxicity. CRC Review of Toxicology. 13: 25-97.

2. Beck, J.F., Donini, J.C. and Maneckjِ, A. (1983) The influence of sulfide and cyanide on axonal function. Toxicology. 26: 37-45.

3. Brown, H.M., Ottoson, D. and Rydqvist, B. (1978) Crayfish stretch receptor: an invesitgation with voltage-clamp on ion-sensitive electrodes. J. Physiol. 284: 155-179.

4. Duan, F.Z. (1959) Maximum permissible concentrations of hydrogen sulfide in the atmosphere. Gig. Sanit. 24 (10) 12.

5. Edwards, C., Terzuolo, C.A. and Washizu, Y. (1963) The effect of changes of the ionic environment upon an isolated crustacean sensory neuron. J. Neurophysiol. 26: 948-957.

6. Higuchi, Y. and Fukamachi, M. (1977) Behavioral studies on toxicity of hydrogen sulfide by means of conditioned avoidance responses in rats. Folia Pharmacol. Jpn. 73 (3) 307.

7. Klie, J.W. and Wilhoner, H.H. (1973) Voltage clamp studies on the stretch response in the neuron of the slowly adapting crayfish stetch receptor. Pflugers ARch. 342: 93-104.

8. Lund, O.E. and Wieland, H. (1966) Pathological-anatomical findings in experimental hydrogen sulfide poisoning (H_2S) - a study on rhesus monkeys. Int. Arch. Gewerhepathol. Bewerbehyg. 22: 46.

9. MacIver, M.B. and Roth, S.H. (1987) Anesthetics produce differential actions on the discharge activity of a single neuron. Europ. J. Pharmacol. 139: 43-52.

10. Roth, S.H. (1980) Molecular and cellular actions of anesthetic agents. Fed. Proceedings. 39: 1595-1599.

11. Sandage, C. (1961) Tolerance criteria for continuous inhalation exposure to toxic material II. Effects on animals of 90-day exposure to H_2S, methyl mercaptan, indole, and skatole. Armed. Ser. Tech. Infor. Agnecy (ASTIA AD 187 797).

12. Sokolove, P.G. and Cooke, I.M. (1971) Inhibition of impulse activity in a sensory neuron by an electrogenic pump. J. Gen. Physiol. 57: 125-163.

13. Tan, K-S and Roth, S.H. (1983) Comparative effects of extracellular ions on the discharge firing activity of an isolated neuron. IRCS Med. Sci. 11: 296-297.

14. Tan, K-S and Roth, S.H. (1984) An in vitro study of copper and copper chelators on the crayfish stretch receptor. Neuropharmacol. 23: 683-689.

15. Van Harreveld, A. (1936) A physiological solution for fresh water crustaceans. Proc. Soc. Exp. Biol. Med. 34: 428-432.

16. Warencyia, M.W., Steele, J.A., Karpinski, E., Reiffenstein, R.J. (1987) Investigation of hydrogen sulfide toxicity in mouse neuroblastoma cells using patch-clamp technique. Soc. Neurosci. Abst. 13: 94.
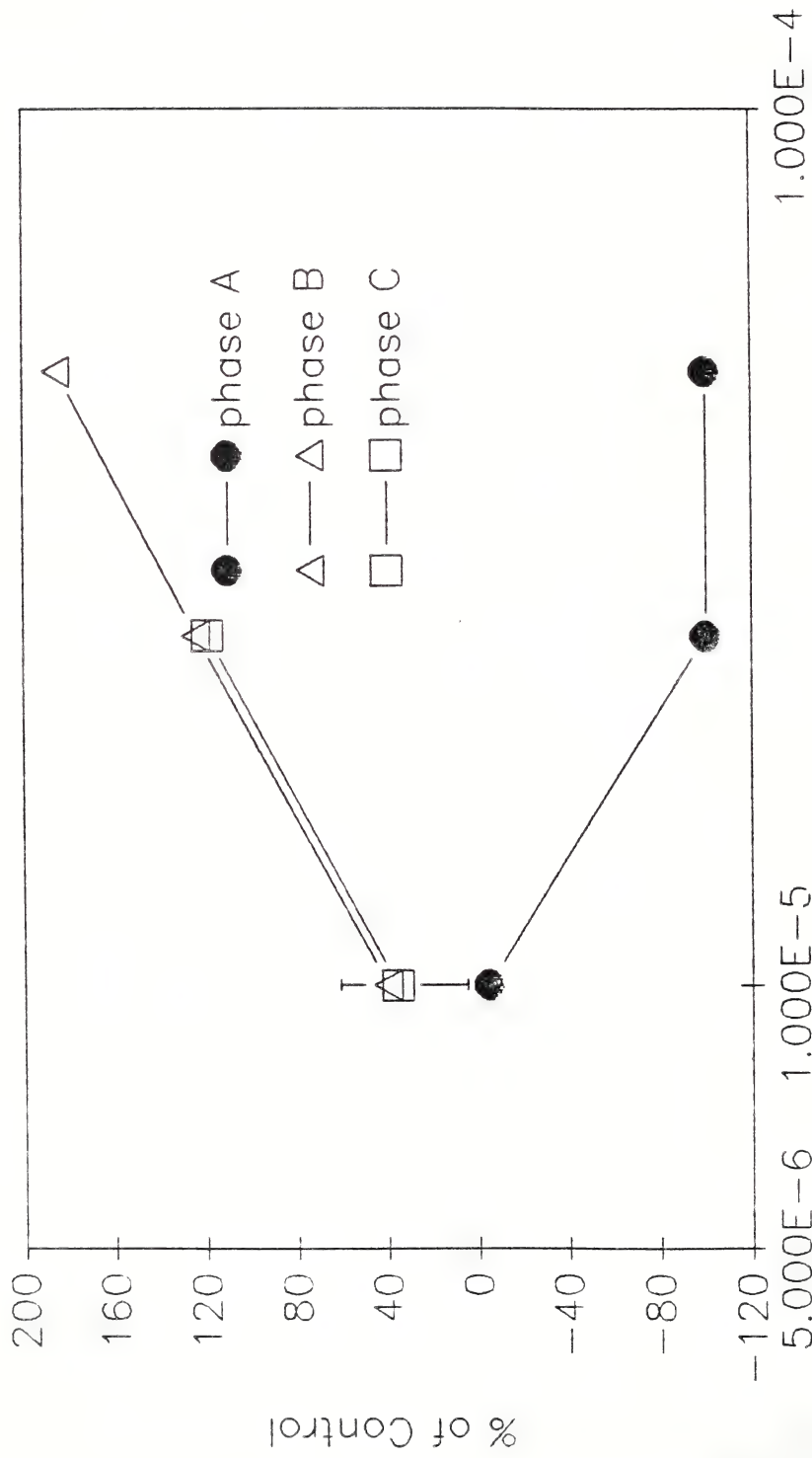
FREQUENCY of FIRING

△——▽ phase B   ●——● phase C

% CONTROL

Na₂S CONCENTRATION

Effect of Na2S on MRO conduction

% Control

100

80

60

40

20

0

−20

5.0E−6   1.0E−5   1.0E−4

Na2S concentration

FREQUENCY of FIRING

% of Control

200 160 120 80 40 0 -40 -80 -120

5.000E-6   1.000E-5   1.000E-4

NaSH Concentration

phase A

phase B

phase C

Distance between Action Potentials

phase A
phase B
phase C

% of Control

NaSH Concentration
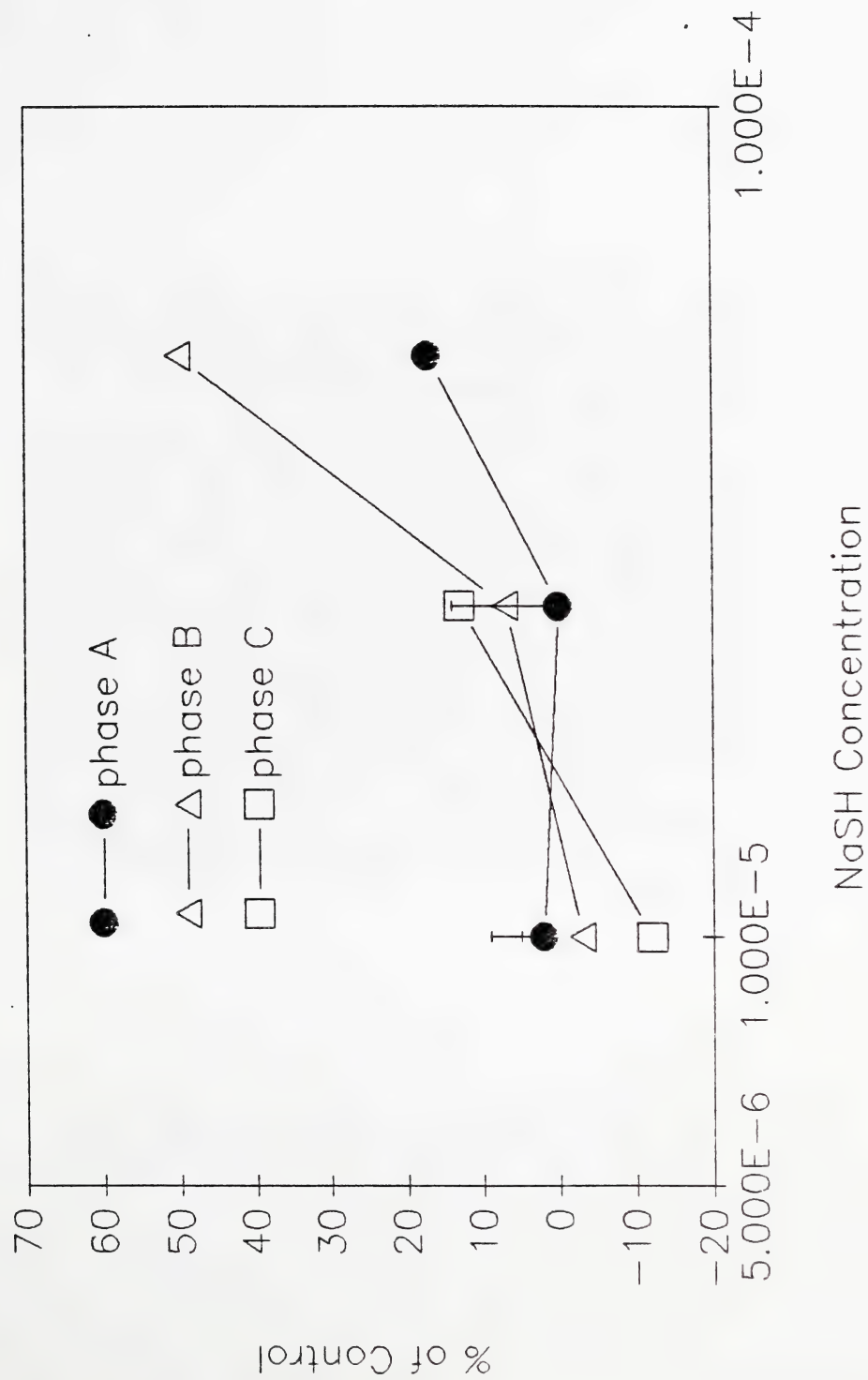
TU-PO-2

EFFECTS OF EXPOSURE TO LOW LEVELS OF HYDROGEN SULFIDE DURING
GESTATION IN DEVELOPING & MATERNAL RATS. L.J. Hayden*,
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   Although the lethal effects of acute exposure to High
levels of hydrogen sulfide (H_2S) have been known for sometime,
very few studies have investigated the effects of chronic
exposure to low levels (less than 100 ppm) of H_2S. This study
is an effort to investigate the effects of low levels of H_2S
on the developing rat pup and maternal rats exposed to H_2S
during gestation.
   Time mated Sprague-Dawley rats were exposed to varying
concentrations of H_2S for 7 hrs from day 7 of gestation until
and including day 20 of gestation. Developing pups were
sacrificed for biochemical analysis of liver and brain tissue
and serum on days 1, 7 and 14 following parturation.
Significant elevations in alkaline phosphatase activity were
observed ($P<0.005$) in brain tissue from all animals exposed to
75 ppm H_2S and in serum from developing pups exposed to 75 ppm
H_2S. Cytochrome P450 levels were not altered in pups exposed
to H_2S but maternal levels were significantly ($P<0.01$)
elevated. These data indicate that low levels of H_2S can
modulate the brain and serum levels of alkaline phosphates and
may induce the synthesis of cytochrome P450 in maternal liver.

436.14

THE EFFECTS OF HYDROGEN SULFIDE EXPOSURE ON AMINO ACID
LEVELS IN THE DEVELOPING RAT CEREBRUM AND CEREBELLUM.

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Humans can be exposed to various chronic levels of
the environmental pollutant, hydrogen sulfide (H_2S). The
exposure occurs both in the work place and by living in
proximity to industries which produce H_2S. Since H_2S
intoxication produces a variety of biochemical changes
in the mature CNS of laboratory animals, it is essential
to examine the effects on the developing CNS which is
extremely sensitive to environmental disturbances.
Timed-pregnant Sprague-Dawley rats were exposed to H_2S
(75 ppm) for seven hours per day from Day 6 postcoitus
until Day 21 postnatal (pn). Entire litters were
euthanized on Days 7, 14 and 21 pn. The cerebrum
(anterior to the optic chiasm) and cerebellum were
removed and stored at $-70°C$. The putative amino acid
neurotransmitters, aspartate, glutamate, glycine,
taurine and GABA were quantitated using precolumn
fluorescence derivatization with OPT and reversed-phase
high performance liquid chromatography. In both regions,
aspartate, glutamate and GABA were significantly reduced
below control levels by Day 21 pn. Taurine which was
initially elevated returned to control levels by Day 21
pn. Glycine levels were unaltered following exposure to
H_2S. (Supported by Alberta Occupational Health and
Safety).

GROWTH OF HUMAN LUNG FIBROBLASTS FOLLOWING
EXPOSURE TO SODIUM SULFIDE. LJ Hayden, SN Faust
and SH Roth, Division of Toxicology, University
of Calgary, Calgary, Alberta, Canada. Sponsor:
FG Biddle

   The growth of fibroblasts derived from fetal
human lung (WI-38) was monitored following acute
exposure of the cells to 250 μM sodium sulfide
(Na_2S). Trypsinized confluent cells were plated
at 10^{-4} cell/well in 1.5 cm multiwell dishes and
allowed to attach for 24 hours. The medium was
then changed to F12 with and without 250 μM Na_2S
(pH 7.4), placed in a sealed environmental
chamber and gassed with 5% CO_2-95% O_2. The cell
cultures were then maintained at 37°C. Medium
from the monolayer cultures was changed after 24
hrs and then every three days thereafter.
Accumulation of protein in each well was
monitored using a quantitative Naphthol Yellow S
stain; DNA was assayed using hydroethidine
fluorescence incorporated into viable cells.
After 24 hr exposure protein content of cultures
incubated with Na_2S was suppressed by 12%.
Cellular DNA accumulation in exposed cells was
30% of control after 24 hrs and then recovered
to a level of 70% of control at 120 hrs. These
studies demonstrate that a single exposure to
Na_2S for 24 hrs inhibits the growth of human
lung fibroblasts. Supported by Alberta
Occupational Health: Heritage Fund.

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MODULATION OF ENZYMATIC ACTIVITY BY LOW LEVELS OF HYDROGEN
SULFIDE IN MATERNAL RATS. <u>L.J. Hayden[*], and S.H. Roth</u>. Div.
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The effects of sub-chronic exposure to low levels of hydro-
gen sulfide (H_2S) on hepatic and neurological alkaline phos-
phatase, cytochrome oxidase and cytochrome P450 activity were
investigated. Time mated Sprague-Dawley rats were exposed to
105 mg/m³ H_2S for 7 hrs from day 7 of gestation until and
including day 20 of gestation. The dams were sacrificed on
day 21 of gestation for biochemical analysis of liver, brain
and serum. Exposed animals were matched with control dams
maintained under the same conditions except for the exclusion
of H_2S from the environment. Liver and brain levels of DNA,
protein, cholesterol, liver P450 and serum alkaline phosphatase
were not significantly altered by exposure to H_2S. However,
cytochrome oxidase activity was significantly depressed by 10%
in whole brain homogenates but was unaffected in hepatic
tissue. Total alkaline phosphatase activity was suppressed by
36% in brain and 34% in liver when assayed in the absence of
added magnesium. This inhibition was reversed when the
alkaline phosphatase was assayed in the presence of magnesium.
Thus, the activity of alkaline phosphatase in brain and liver,
and cytochrome oxidase in brain was decreased when dams were
not permitted to recover from exposure to low levels of H_2S.
(Supported by Alberta Occupational Health/Heritage Fund.)

INHIBITION OF OXYTOCIN INDUCED UTERINE CONTRACTION FOLLOWING LOW DOSE ACUTE EXPOSURE TO SODIUM SULFIDE. <u>K.J. Franklin</u>*, <u>L.J. Hayden</u>*, <u>S.H. Roth</u> and <u>G.J. Moore</u>, Departments of Pharmacology & Therapeutics & Medical Biochemistry, University of Calgary, Alberta T2N 4N1

Sub-chronic exposure to low levels of hydrogen sulfide (H_2S) increased the length of parturition time in Sprague-Dawley rats. In order to determine if H_2S directly effects uterine tissue, a uterine muscle contraction assay was performed following exposure of the tissue to varying concentrations of sodium sulfide (Na_2S). Muscle tissue which had been characterized for response to oxytocin (OXY) and angiotensin (ANG) was bathed for 10 minutes in oxygenated DeJolon's buffer containing Na_2S. The Na_2S containing buffer was washed from the chamber with DeJolon's buffer, continuous oxygenation restored and the tissue immediately challenged with either OXY or ANG. Sodium sulfide at 1.5 μM produced a suppression of the OXY induced contraction by 43 percent after a 10 minute incubation. A 10 fold increase in Na_2S (15 μM) produced a similar response while 0.15 μM Na_2S produced a marginal suppression of contraction. The contraction response to OXY returned to normal within five minutes of removal of the Na_2S. Exposure of the muscle tissue did not effect ANG contractions. Thus, Na_2S directly and reversibly inhibited OXY induced contractions in uterine muscle. (Supported by Alberta Occupational Health/Heritage Fund and Alberta Heart Foundation)

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HYDROGEN SULFIDE AND ITS EFFECTS ON THE MORPHOLOGICAL
DEVELOPMENT OF THE RAT CEREBELLUM.

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Hydrogen sulfide (H_2S) is an environmental pollutant
which produces deleterious effects on the developing
central nervous system. Pregnant rats (Sprague-Dawley)
were exposed to H_2S (75 ppm) for seven hours per day
from Day 7 until Day 21 postcoitus in an environment
chamber. Entire litters were euthanized on Day 7 and Day
14 postnatal and the cerebella removed. Purkinje and
granule cells were quantitated along the length of the
primary fissure. The quantitation demonstrated a signi-
ficant increase in the density of the treated Purkinje
cells (cells/mm) which would result in the Purkinje
cells attempting to establish their respective dendritic
fields in a more confined space. The ratio of granule
cells to Purkinje cells was significantly lower in the
treated group at both ages, which suggests that fewer
parallel fibres were available to individual Purkinje
cells. The finding that prenatal hydrogen sulfide
treatment produces structural alterations in the
cerebellum suggests that many other neuronal populations
may also be at risk.

407.1

LOW DOSE HYDROGEN SULFIDE AND ITS EFFECTS ON THE DENDRITIC
ARBORIZATION OF DEVELOPING CEREBELLAR PURKINJE CELLS.
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Anatomy and of Pharmacology and Therapeutics, and Div. of
Toxicology, Faculty of Medicine, University of Calgary,
Calgary, Alberta, Canada. T2N 4N1.
Hydrogen sulfide (H_2S) is an environmental pollutant
which can produce severe effects on the adult CNS;however,
little is known as to its effects on the developing CNS.
Pregnant rats (Sprague-Dawley) were exposed to either 20
pmm or 50 pmm H_2S for 7 hours per day in an environment
chamber from Day 7 postcoitus until Day 21 postnatal.
Controls were similarly treated including placement in an
environmental chamber flushed with room air. On Day 21
postnatal, representative pups from each litter were
euthanized, the cerebella removed and processed for Golgi
staining. Ten complete Purkinje cells were selected from
each group and analysed, using vertex analysis (Berry &
Finn, Proc. R. Soc. Lond. B. 221:321, 1984). At both
exposure concentrations, there was a significant increase
in vertex path length suggesting an increased distance
between each new generation of branches. New growth
occurred toward the pial surface and internally but was
restricted laterally. The treated cells exhibited an
unusually high non-random growth pattern. In summary,
both treatments produced significant growth changes in
the dendritic arborization.

CHARACTERIZATION OF HUMAN LUNG FIBROBLAST GROWTH FOLLOWING EXPOSURE TO SODIUM SULFIDE, <u>L.J. Hayden</u>[*], H. Cheng[*] and <u>S.H. Roth</u>, Division of Toxicology, University of Calgary, Alberta, Canada T2N 4N1

Fetal human lung fibroblast (WI-38) growth was monitored following acute exposure of the cells to 250 μM sodium sulfide (Na$_2$S). Trypsinized confluent cells were plated at 10^{-4} cell/well in 1.5 cm multiwell dishes and allowed to attach for 24 hours. The medium was then changed to F12 with and without 250 μM Na$_2$S (pH 7.4), placed in a sealed environmental chamber and gassed with 5% CO$_2$-95% O$_2$. The cell cultures were then maintained at 37°C. Medium from the monolayer cultures was changed after 24 hrs and then every three days thereafter. Accumulation of protein in each well was monitored using a quantitative Naphthol Yellow S stain; DNA was assayed using hydroethidine fluorescence incorporated into viable cells. After 24 hr exposure protein content of cultures incubated with Na$_2$S was suppressed by 12%. Cellular DNA accumulation in exposed cells was 30% of control after 24 hrs and then recovered to a level of 70% of control at 120 hrs. These studies demonstrate that a single exposure to Na$_2$S for 24 hrs inhibits the growth of human lung fibroblasts.

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THE EFFECTS OF LOW CONCENTRATIONS OF HYDROGEN SULPHIDE
ON THE DISCHARGE ACTIVITY OF AN ISOLATED SENSORY
NEURON. S.H. Roth, H. Mathison , L.J. Hayden and
R.S. Hannah. Dept. of Pharmacology, Division of
Toxicology, University of Calgary, Alberta T2N 4N1.

Hydrogen sulphide (H_2S) is an industrial and
environmental pollutant. The major target organ for
toxicity appears to be the central nervous system.
Acute exposures to H_2S have been associated with signs
of cerebral and extrapyramidal damage, facial
paralysis, prolonged reaction times, absent or abnormal
reflexes, loss of short term memory, depression and
epileptic-like seizures which are all neural in origin.
The present study determined the effects of both
gaseous H_2S and sodium sulphide (the non-volatile salt
of H_2S) on the discharge activity of the isolated
crayfish neuron. The effects of both agents were
complex, concentration and time dependent. At
concentrations of 1 to 30 μM both agents produced a
triple phased response (depression, excitation and
return towards control levels of activity). Conduction
velocities did not change significantly at low
concentrations. At higher concentrations the effects
were often irreversible. It is evident acute exposures
to low concentrations of H_2S can alter neuronal
function. Supported by Alberta Occupational Health and
Safety Heritage Program.

HUMAN LUNG FIBROBLAST CYTOTOXICITY FOLLOWING ACUTE
SODIUM SULFIDE EXPOSURE. L.J. Hayden; A.C. Pui &
S.H. Roth. Division of Toxicology, University of
Calgary, Calgary, Canada T2N 4N1.

It has been well documented that environmental
and pharmaceutical compounds can induce leakage of
cell content which is often used as a measure of
cell membrane permeability and cytotoxicity.
However, these procedures do not reflect the more
subtle changes in either specific cell function or
growth. In the present studies, the growth of
human lung fibroblasts (WI-38) was monitored
following acute exposures to low concentrations of
Na_2S and $NaHSO_3$ using measurements of protein and
DNA accumulation. Fibroblast cytotoxicity
following exposure to Na_2S or $NaHSO_3$ was
determined by analysis of lactate dehydrogenase
(LDH) leakage from the cells. It was observed that
the loss of LDH from confluent cells was
concentration dependent; maximum leakage occurred
at 2-4 hours followed by a return to control
levels at 8 hours. Analysis of cellular growth was
continued for 16 days following a 24 hour exposure
to 10 μM Na_2S or $NaHSO_3$. After 48 hours, the
protein content was 80% of control, and DNA
accumulation was approximately 40% of control. The
reduction of protein content was similar for the
cells exposed to both agents, however DNA
accumulation was less affected in the presence of
$NaHSO_3$ compared to Na_2S. These studies demonstrate
that acute exposure to low concentrations of Na_2S
can be cytotoxic as reflected by the loss of
cellular protein and DNA, however growth of the
surviving cells appears normal. Supported by the
Alberta Occupational Health & Safety.

A Relationship Between Hydrogen Sulfide Exposure and Taurine Levels in Maternal Rats. R.S. Hannah, R. Bennington and S.H. Roth, Division of Toxicology, Depts of Anatomy and Pharmacology/Therapeutics, University of Calgary, Calgary, AB, T2N 4N1

We have previously demonstrated that exposure to low concentrations of hydrogen sulfide (H_2S) produces a significant increase in the level of taurine in the developing rat CNS which return to control values at the same approximate time as the establishment of the blood-brain barrier (Hannah et al. Neurosci. Lett. 99:323-327, 1989). The purpose of this study was to establish if this observed elevation was the result of increased maternal plasma levels of taurine. Time-pregnant Sprague-Dawley rats were exposed to H_2S (50 ppm) for seven hours per day from Day 6 postcoitus until Day 21 postnatal. Maternal blood plasma was sampled on the day of parturition and Day 21 post-partum. Taurine levels were quantitated using precolumn fluorescence derivitization with OPT and reversed-phase high performance liquid chromatography. Taurine levels in materbal plasma were found to be increased at both sample times. Therefore, the high initial levels of taurine observed in the pup CNS may have been maternal in origin and not endogenously produced. Since taurine is known to have an attenuated action on toxic compounds, the rise in maternal taurine levels may represent a protective mechanism driven by the H_2S exposure.